Working with Gene Lists and Over-representation Analysis

MBV-INF4410

Tuesday, September 15th, 2009

Ian Donaldson

http://donaldson.uio.no

This talk is a remix of two talks presented in 2009 at the Canadian Bioinformatics Workshops by Gary Bader and Quaid Morris. Many thanks to Gary, Quaid and the CBW for making this material available.

Ian Donaldson, September 14th

http://baderlab.org
http://morrislab.med.utoronto.ca/
http://www.bioinformatics.ca/workshops/2009/course-content
(see Interpreting Gene Lists from -omics Studies.
Module 1: Introduction to gene lists (Chair: Gary Bader) and
Module 2: Finding over-represented gene functions in gene lists (Chair: Quaid Morris)

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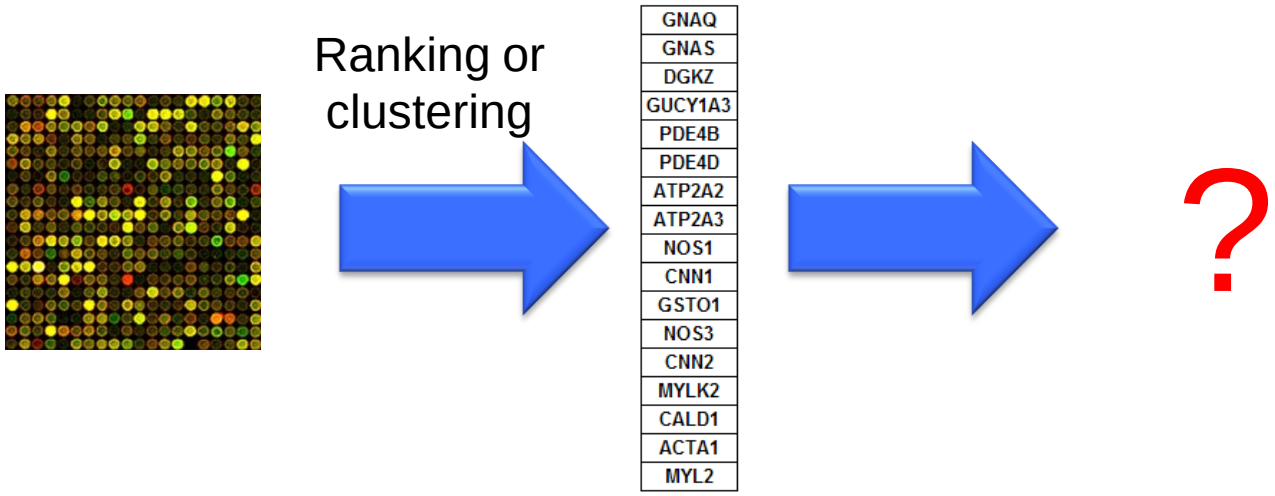
Gene Lists Overview

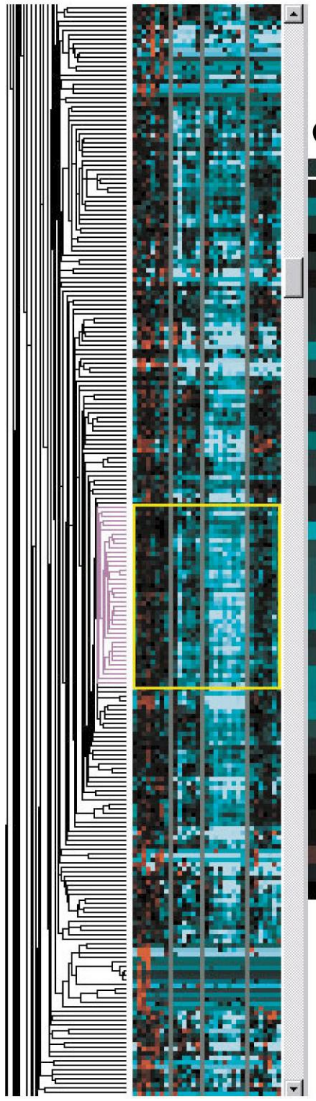
- Interpreting gene lists
- Gene attributes
  - Gene Ontology
    - Ontology Structure
    - Annotation
  - BioMart + other sources
- Gene identifiers and mapping
- Part 2: Network Introduction

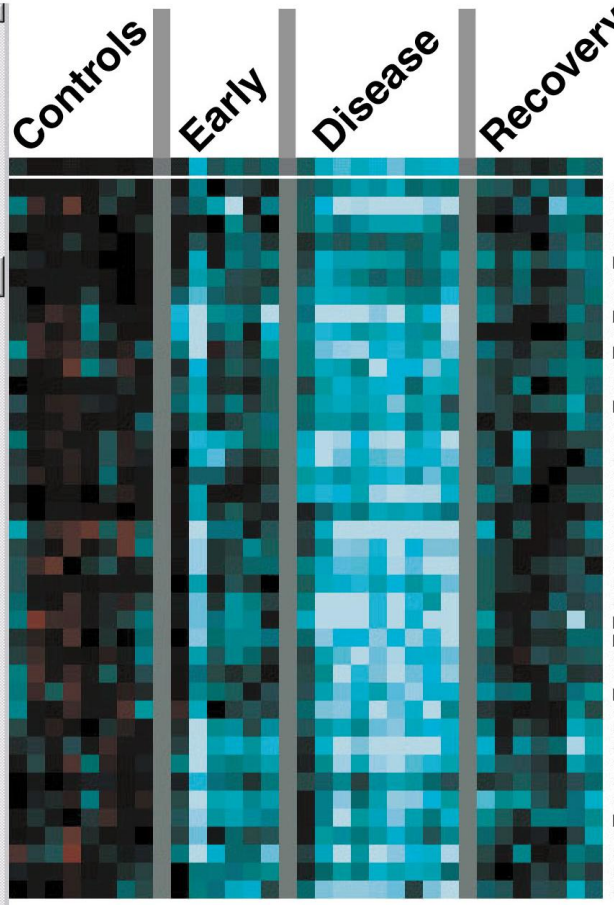
Interpreting Gene Lists

- My cool new screen worked and produced 1000 hits! …Now what?
- Genome-Scale Analysis (Omics)
  - Genomics, Proteomics



Cardiomyopathy: Downregulated Genes





BLAST Definitions

"Homologous to sp O07021: PRE-MRNA SPLICING FACTOR SF2, P32 SUBUNIT PRECURSOR (GCIQ-R PROTEIN)
"Mus musculus proteasome activator PA28 alpha subunit mRNA, complete cds"
"Mus musculus cdc37 homolog mRNA, complete cds"
"Mus musculus ornithine decarboxylase antizyme gene, complete cds"
M.musculus mRNA for carnitine acetyltransferase
"Homologous to sp O00779: CALCIUM-TRANSPORTING ATPASE SARCOPLASMIC RETICULUM TYPE (EC 3.6.1.3
"Homologous to sp P11507: CALCIUM-TRANSPORTING ATPASE ENDOPLASMIC RETICULUM TYPE (EC 3.6.1.38)
M.musculus ENO3 mRNA for enolase beta subunit
"Homologous to sp P47858: 6-PHOSPHOFRUCTOKINASE, MUSCLE TYPE (EC 2.7.1.11) (PHOSPHOFRUCTOKINAS
Homologous to sp P23327: SARCOPLASMIC RETICULUM HISTIDINE-RICH CALCIUM-BINDING PROTEIN PRECURS
"Mouse AE3 mRNA, complete cds"
"M.musculus glucose transporter 2 mRNA, complete cds"
Mus musculus aspartate aminotransferase gene 5'-flank and exon 1
"Mus musculus thioredoxin-dependent peroxide reductase (tpx) mRNA, complete cds"
"Homologous to sp P47858: 6-PHOSPHOFRUCTOKINASE, MUSCLE TYPE (EC 2.7.1.11) (PHOSPHOFRUCTOKINAS
"Homologous to sp P11508: CALCIUM-TRANSPORTING ATPASE SARCOPLASMIC RETICULUM TYPE (EC 3.6.1.3
"Homologous to sp P35434: ATP SYNTHASE DELTA CHAIN, MITOCHONDRIAL PRECURSOR (EC 3.6.1.34)."
"Mus musculus F1F0ATP synthase complex E subunit (Atp5k) gene, complete cds"
"Mus musculus NAD(H)-specific isocitrate dehydrogenase gamma subunit precursor, mRNA, complete
"M.musculus gene for dodecenoyl-CoA delta-isomerase, exons 1 and 2"
"Mus musculus cytochrome c oxidase subunit VIII-H precursor (COX8H) mRNA, complete cds"
"Homologous to sp P35745: ACYLPHOSPHATASE, MUSCLE TYPE ISOZYME (EC 3.6.1.7) (ACYLPHOSPHATE PH
"Mus musculus CD-1 cardiac troponin I mRNA, complete cds"
"Homologous to sp P00566: CREATINE KINASE, M CHAIN (EC 2.7.3.2) (HU-2 PROTEIN)."
Mouse mRNA for protein with homology to transition protein 2 (TP2)
Mus musculus Selenium-binding liver protein mRNA
"Mus musculus (clone MAR1) aldose reductase mRNA, complete cds"
"Mus musculus vascular endothelial growth factor B 186 (VEGF-B) precursor, mRNA, complete cds"
M.musculus mRNA for NADP transhydrogenase
"Mus musculus aldehyde dehydrogenase (ALDH2) mRNA, nuclear gene encoding mitochondrial protein
"Mouse cytosolic epoxide hydrolase mRNA, complete cds"
"Mus musculus 129SV carnitine palmitoyltransferase II mRNA, complete cds"
"Mus musculus medium-chain acyl-CoA dehydrogenase mRNA, complete cds"
"Mus musculus long-chain acyl-CoA dehydrogenase mRNA, complete cds"
"Mus musculus very-long chain acyl-CoA dehydrogenase mRNA, partial cds"
Mouse muscle creatine kinase mRNA (EC 2.7.3.2)
"Mus musculus isocitrate dehydrogenase mRNA, complete cds"
"Mus musculus long chain fatty acyl CoA synthetase mRNA, complete cds"
"Mus musculus sterol carrier protein-2 (SCP-2) gene, complete cds"
"Mouse alpha-tubulin isotype M-alpha-4 mRNA, complete cds"

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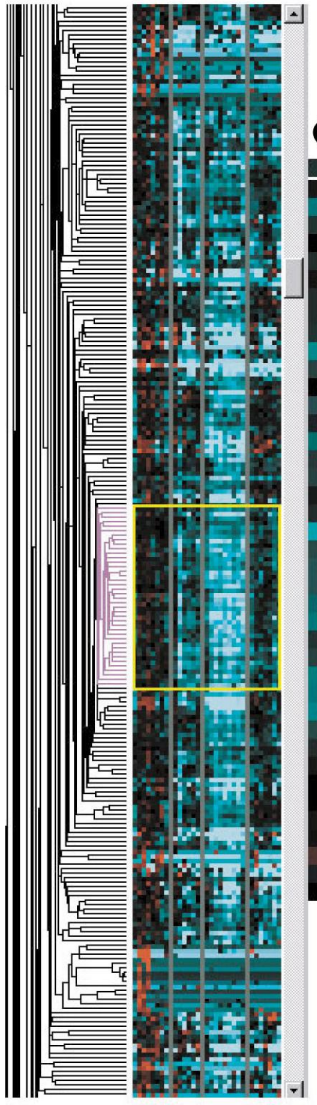
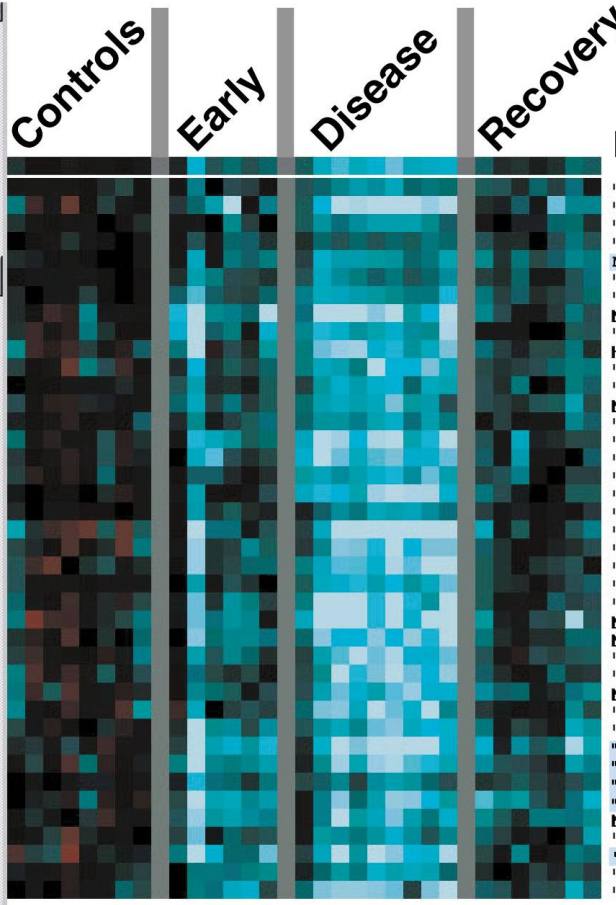
GenMAPP.org

Cardiomyopathy: Downregulated Genes





Anecdote vs. significance?

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"Mus musculus sterol carrier protein-2 (SCP-2) gene, complete cds"
"Mouse alpha-tubulin isotype M-alpha-4 mRNA, complete cds"

Fatty Acid Degradation?
Other pathways / processes?

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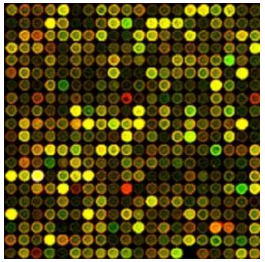
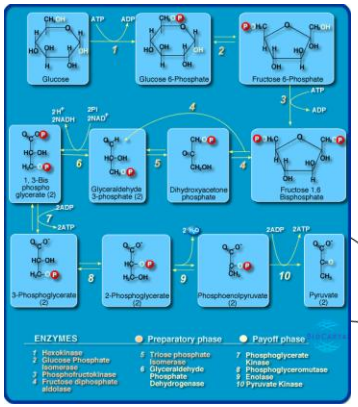
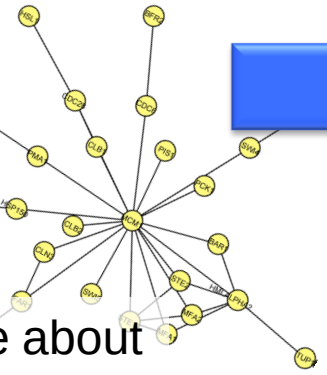
  - Genomics, Proteomics



Ranking or clustering

GNAQ
GNAS
DGKZ
GUCY1A3
PDE4B
PDE4D
ATP2A2
ATP2A3
NOS1
CNN1
GSTO1
NOS3
CNN2
MYLK2
CALD1
ACTA1
MYL2





Prior knowledge about cellular processes

Analysis tools

Eureka! New heart disease gene!

Where Do Gene Lists Come From?

- Molecular profiling e.g. mRNA, protein
  - Identification → Gene list
  - Quantification → Gene list + values
  - Ranking, Clustering (biostatistics)
- Interactions: Protein interactions, Transcription factor binding sites (ChIP)
- Genetic screen e.g. of knock out library
- Association studies (Genome-wide)
  - Single nucleotide polymorphisms (SNPs)
  - Copy number variants (CNVs)

Other examples?

What Do Gene Lists Mean?

- Biological system: complex, pathway
- Similar gene function e.g. protein kinase
- Similar cell or tissue location
- Chromosomal location (linkage, CNVs)

Biological Questions

- Step 1: What do you want to accomplish with your list (hopefully part of experiment design! ☺)
  - Summarize biological processes or other aspects of gene function
  - Controller for a process (TF)
  - Find new pathways or new pathway members
  - Discover new gene function
  - Correlation to a disease or phenotype (candidate gene prioritization)
  - Differential analysis – what's different between samples?

Biological Answers

- Computational analysis methods
  - Gene set analysis: summarize
  - Gene regulation network analysis
  - Pathway and network analysis
  - Gene function prediction

- But first! Gene list basics…

Gene Lists Overview

- Interpreting gene lists
- Gene attributes
  - Gene Ontology
    - Ontology Structure
    - Annotation
  - BioMart + other sources
- Gene identifiers and mapping

Gene Attributes

- Available in databases
- Function annotation
  - Biological process, molecular function, cell location
- Chromosome position
- Disease association
- DNA properties
  - TF binding sites, gene structure (intron/exon), SNPs
- Transcript properties
  - Splicing, 3’ UTR, microRNA binding sites
- Protein properties
  - Domains, secondary and tertiary structure, PTM sites
- Interactions with other genes

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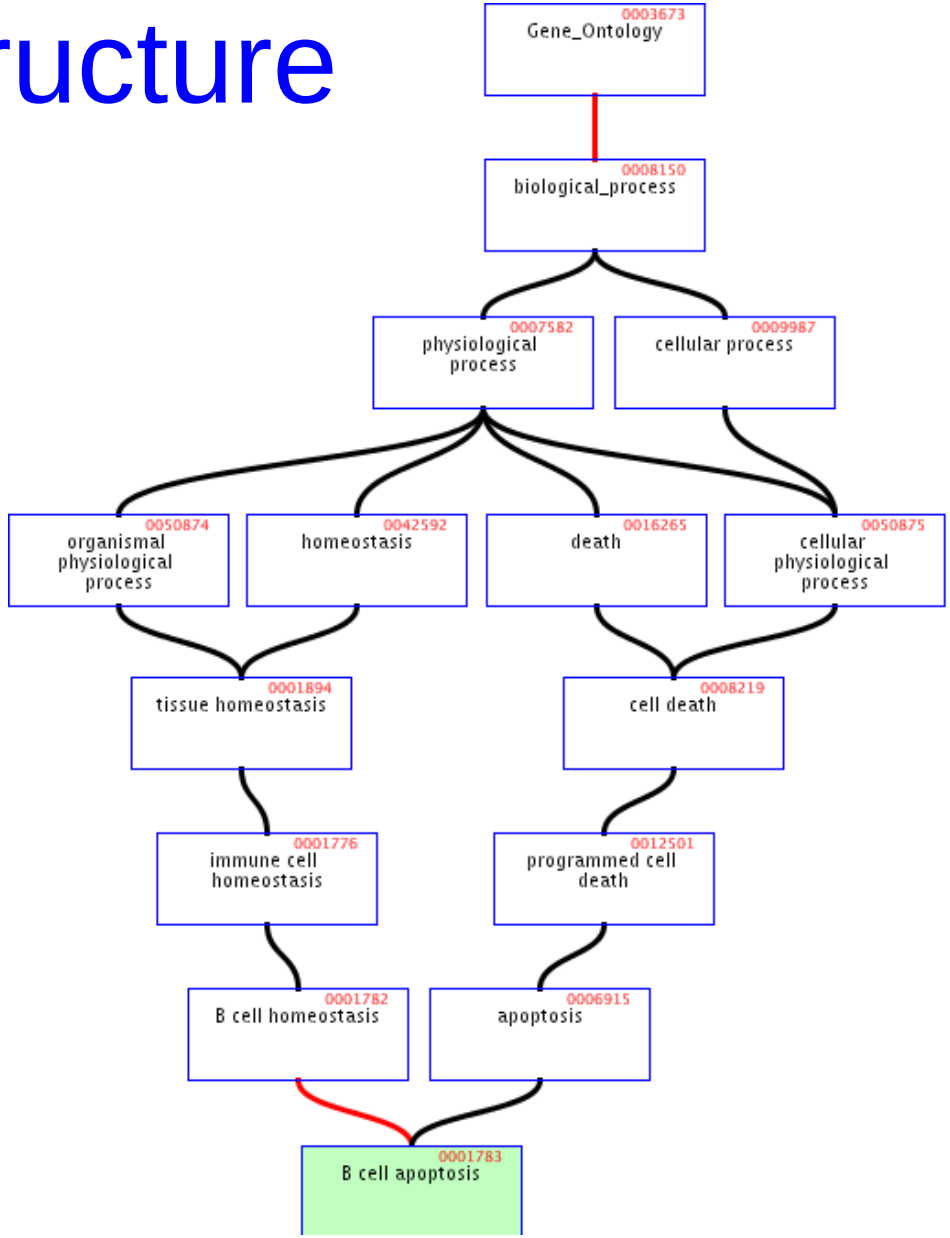
What is the Gene Ontology (GO)?

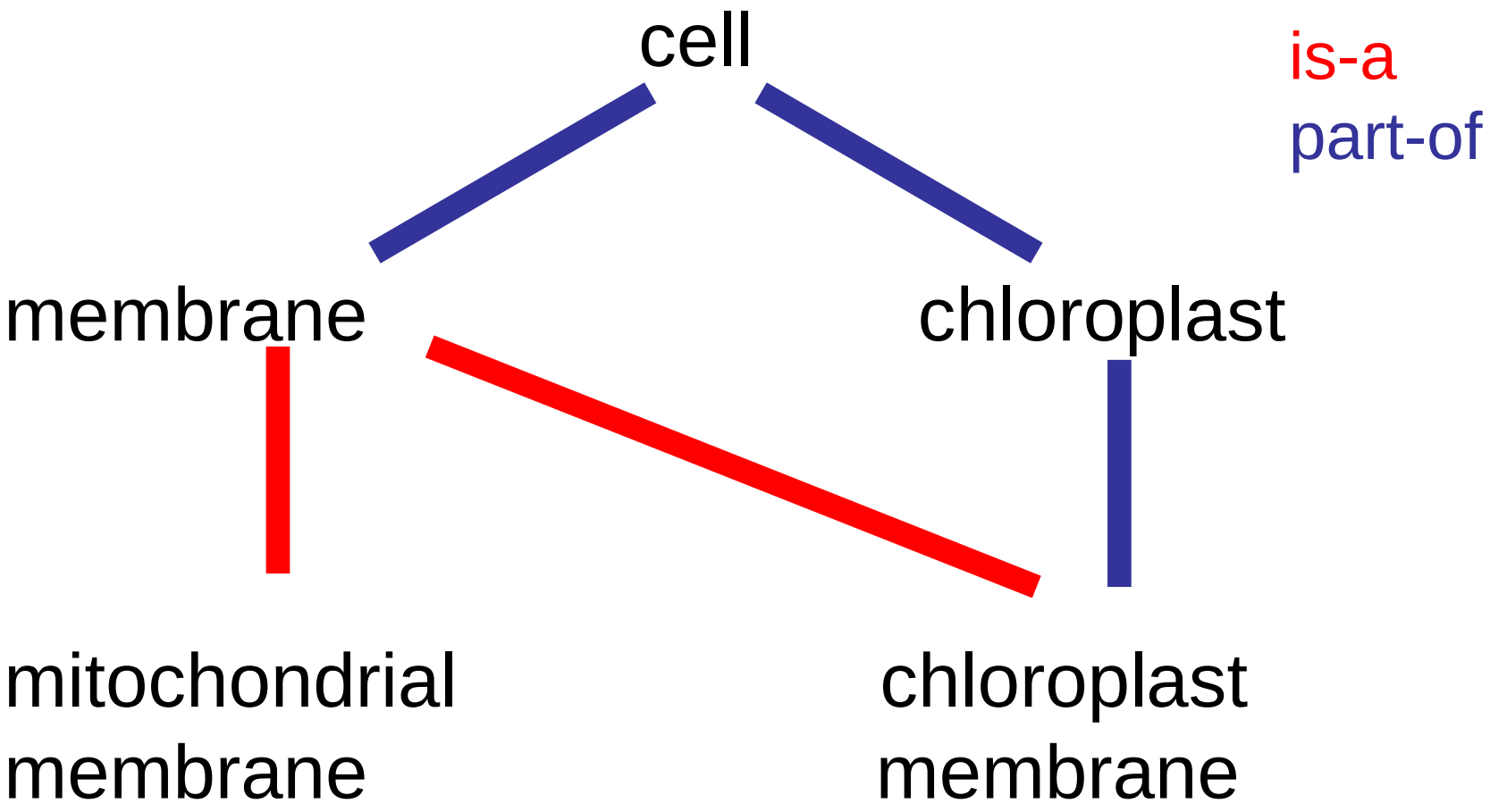
- Set of biological phrases (terms) which are applied to genes:
  - protein kinase
  - apoptosis
  - membrane
- Ontology: A formal system for describing knowledge

GO Structure

- Terms are related within a hierarchy
  - is-a
  - part-of
- Describes multiple levels of detail of gene function
- Terms can have more than one parent or child



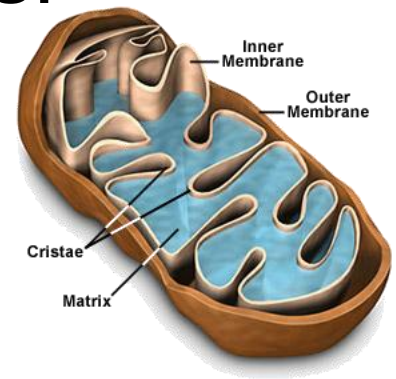
GO Structure

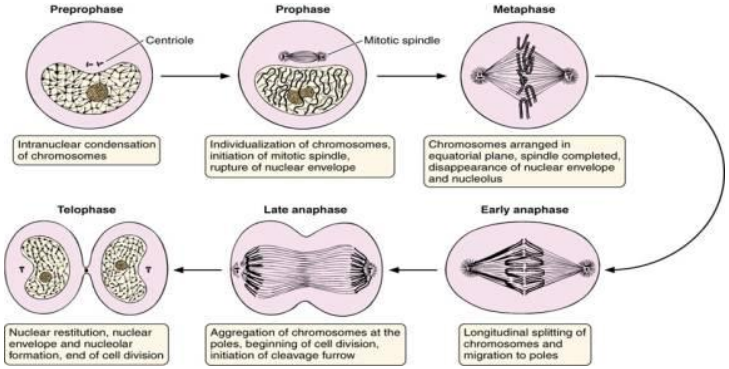


Species independent. Some lower-level terms are specific to a group, but higher level terms are not

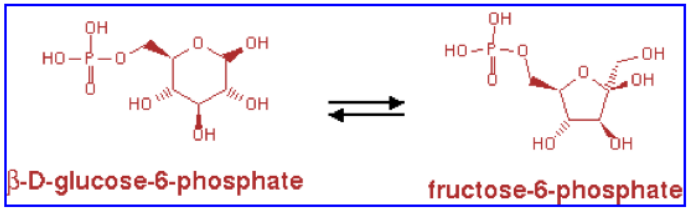
What GO Covers?

- GO terms divided into three aspects:
  - cellular component
  - molecular function
  - biological process





Cell division



glucose-6-phosphate isomerase activity

Terms

- Where do GO terms come from?
  - GO terms are added by editors at EBI and gene annotation database groups
  - Terms added by request
  - Experts help with major development
  - 27734 terms, 98.9% with definitions.
    - 16731 biological_process
    - 2385 cellular_component
    - 8618 molecular_function
    - As of July 6, 2009

Annotations

- Genes are linked, or associated, with GO terms by trained curators at genome databases
  - Known as ‘gene associations’ or GO annotations
  - Multiple annotations per gene
- Some GO annotations created automatically

Annotation Sources

- Manual annotation
  - Created by scientific curators
    - High quality
    - Small number (time-consuming to create)
- Electronic annotation
  - Annotation derived without human validation
    - Computational predictions (accuracy varies)
    - Lower ‘quality’ than manual codes
- Key point: be aware of annotation origin

For your information

Evidence Types

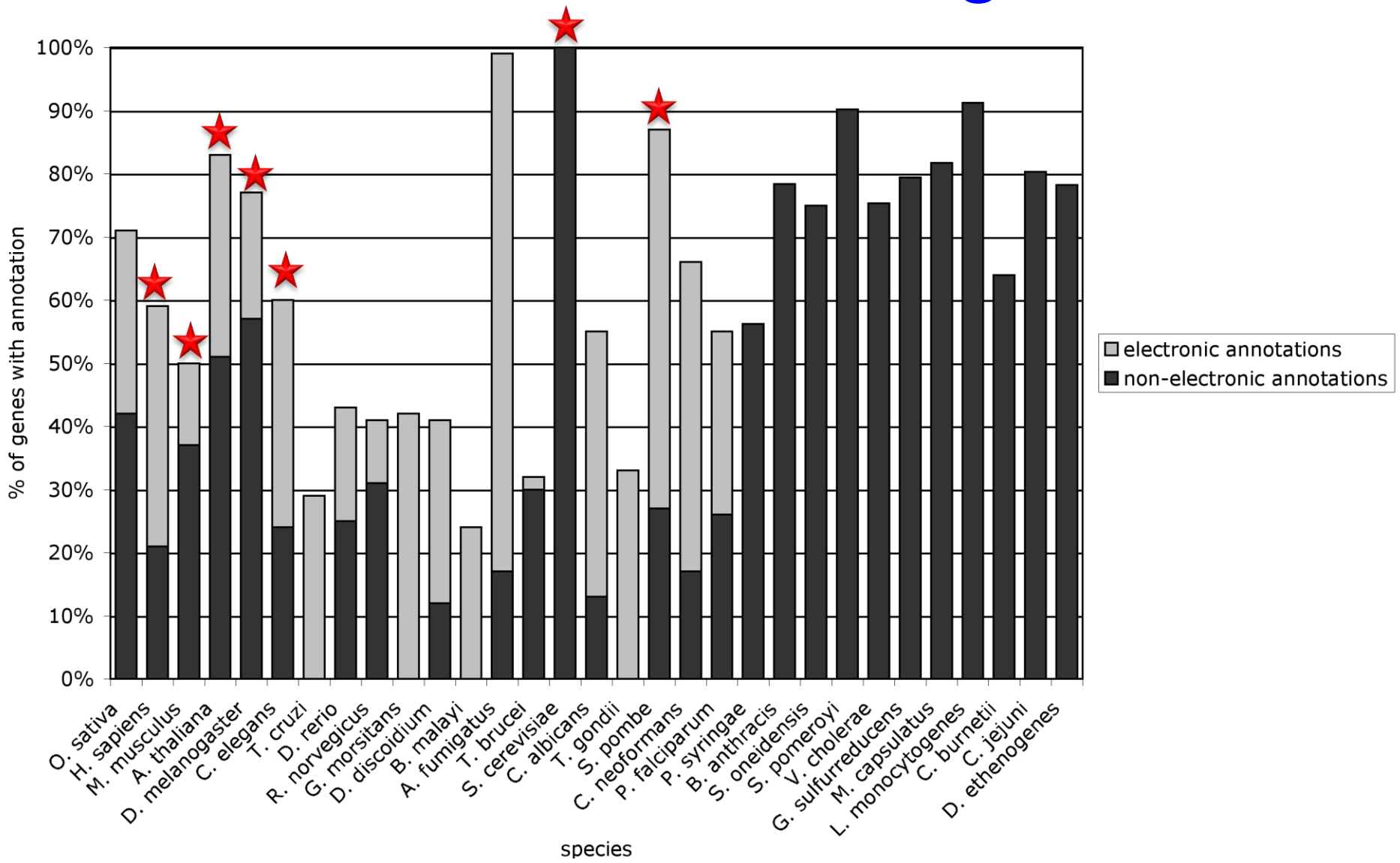
- **ISS:** Inferred from Sequence/Structural Similarity
- **IDA:** Inferred from Direct Assay
- **IPI:** Inferred from Physical Interaction
- **IMP:** Inferred from Mutant Phenotype
- **IGI:** Inferred from Genetic Interaction
- **IEP:** Inferred from Expression Pattern
- **TAS:** Traceable Author Statement
- **NAS:** Non-traceable Author Statement
- **IC:** Inferred by Curator
- **ND:** No Data available

- **IEA:** Inferred from electronic annotation

Species Coverage

- All major eukaryotic model organism species
- Human via GOA group at UniProt
- Several bacterial and parasite species through TIGR and GeneDB at Sanger
- New species annotations in development

Variable Coverage



Lomax J. Get ready to GO! A biologist's guide to the Gene Ontology. Brief Bioinform. 2005 Sep;6(3):298-304.

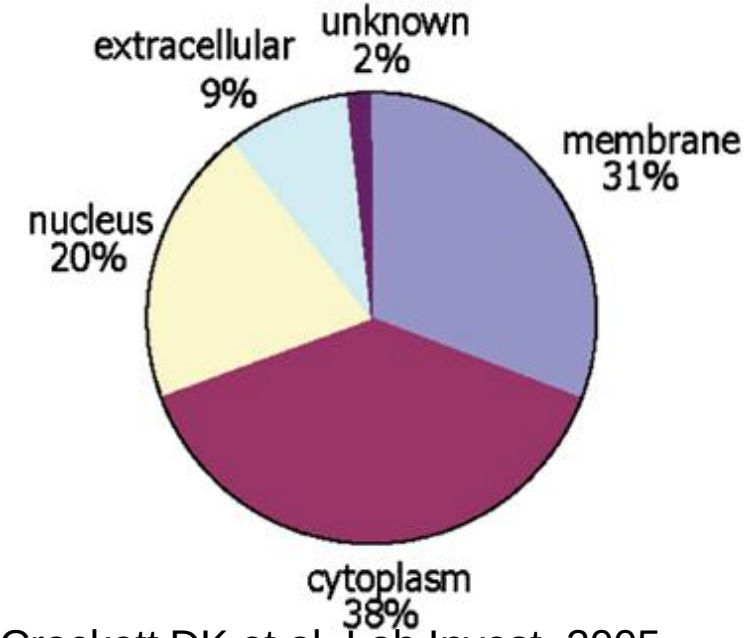
For your information

Contributing Databases

- Berkeley *Drosophila* Genome Project (BDGP)
- dictyBase (*Dictyostelium discoideum)*
- FlyBase (*Drosophila melanogaster)*
- GeneDB (*Schizosaccharomyces pombe*, *Plasmodium falciparum*, *Leishmania major* and *Trypanosoma brucei)*
- UniProt Knowledgebase (Swiss-Prot/TrEMBL/PIR-PSD) and InterPro databases
- Gramene (grains, including rice, *Oryza*)
- Mouse Genome Database (MGD) and Gene Expression Database (GXD) (*Mus musculus)*
- Rat Genome Database (RGD) (*Rattus norvegicus)*
- Reactome
- *Saccharomyces* Genome Database (SGD) (*Saccharomyces cerevisiae)*
- The *Arabidopsis* Information Resource (TAIR) (*Arabidopsis thaliana)*
- The Institute for Genomic Research (TIGR): databases on several bacterial species
- WormBase (*Caenorhabditis elegans)*
- Zebrafish Information Network (ZFIN): (*Danio rerio)*

GO Slim Sets

- GO has too many terms for some uses
  - Summaries (e.g. Pie charts)
- GO Slim is an official reduced set of GO terms
  - Generic, plant, yeast

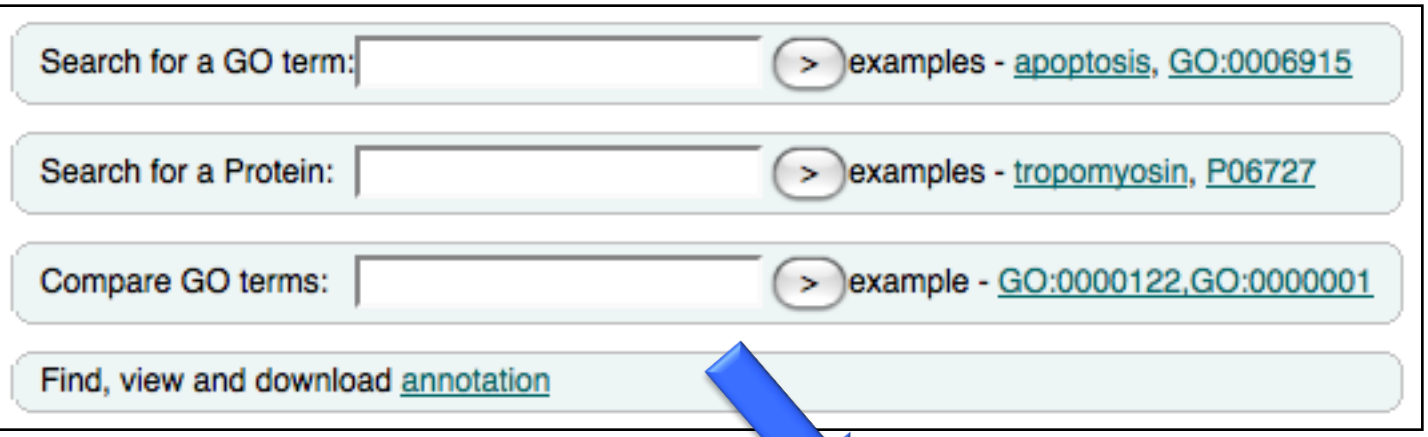


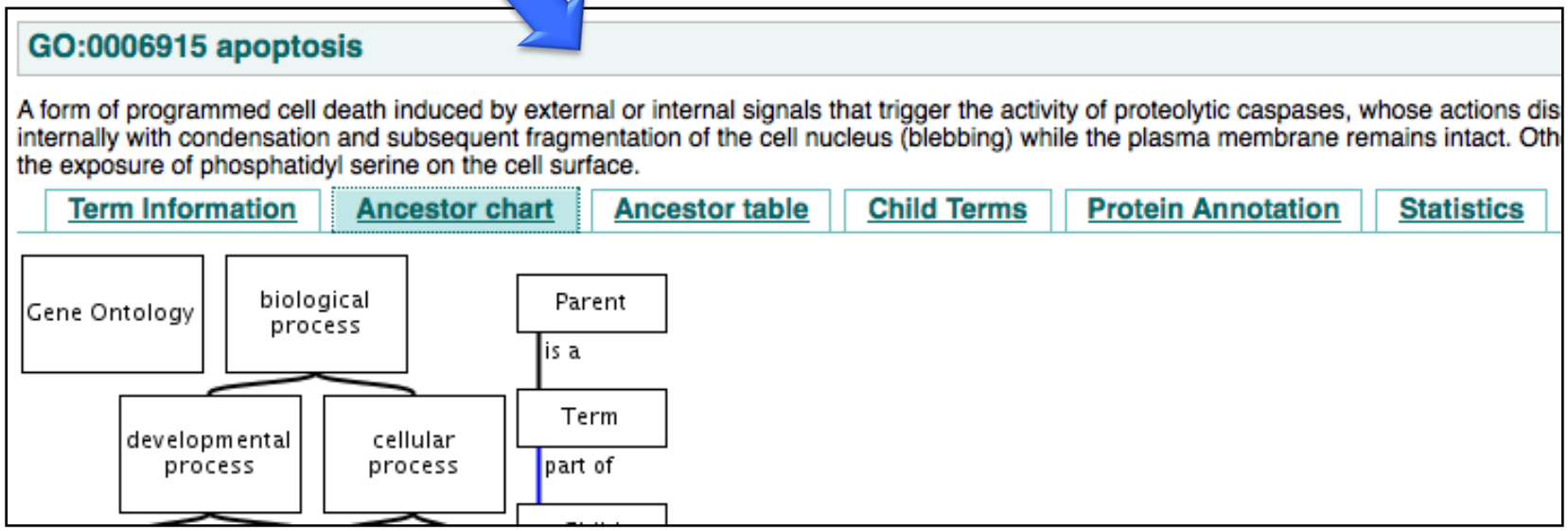
Crockett DK et al. Lab Invest. 2005 Nov;85(11):1405-15

GO Software Tools

- GO resources are freely available to anyone without restriction
  - Includes the ontologies, gene associations and tools developed by GO
- Other groups have used GO to create tools for many purposes
  - http://www.geneontology.org/GO.tools

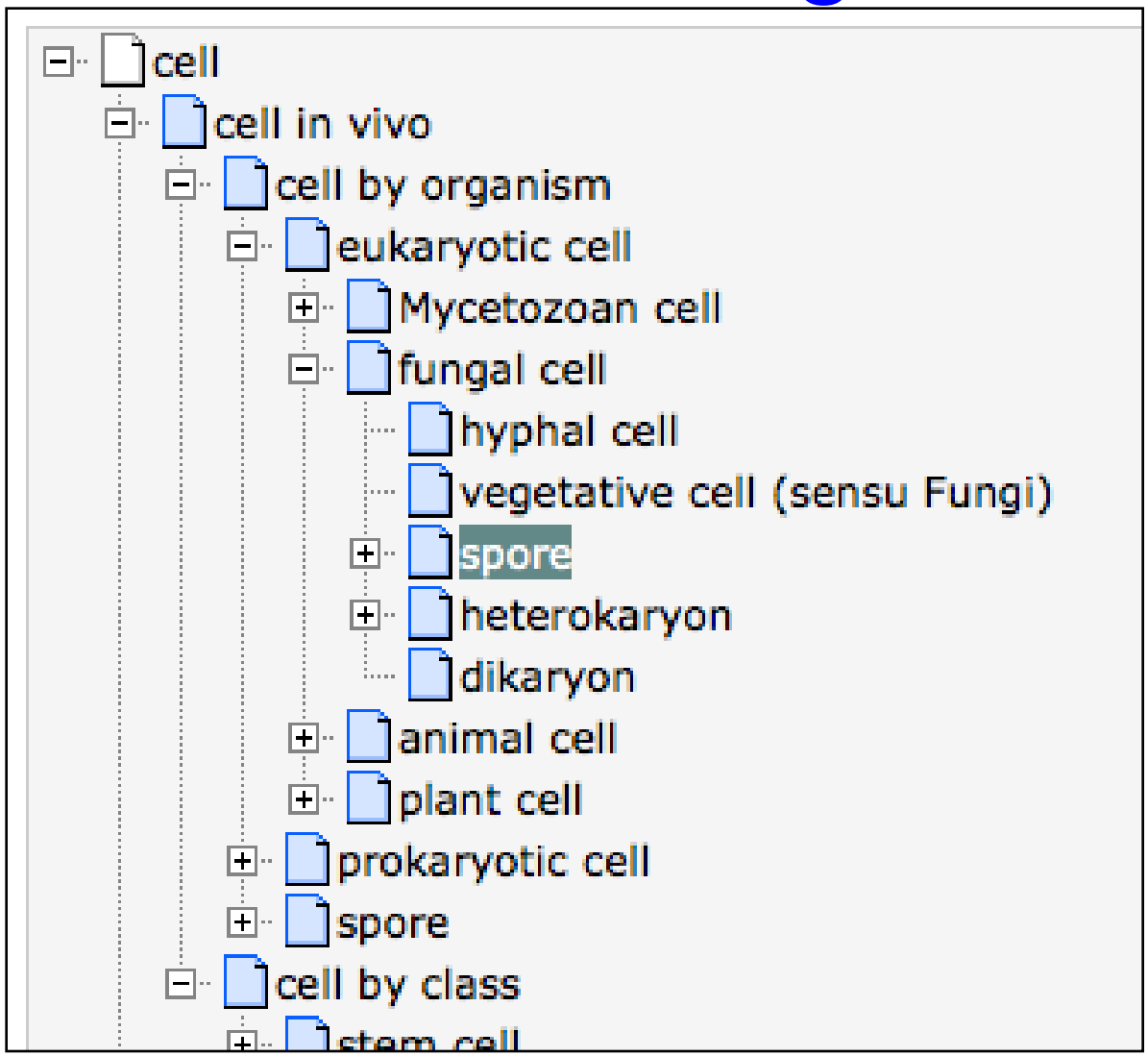
Accessing GO: QuickGO





http://www.ebi.ac.uk/ego/

Other Ontologies



http://www.ebi.ac.uk/ontology-lookup

Gene Attributes

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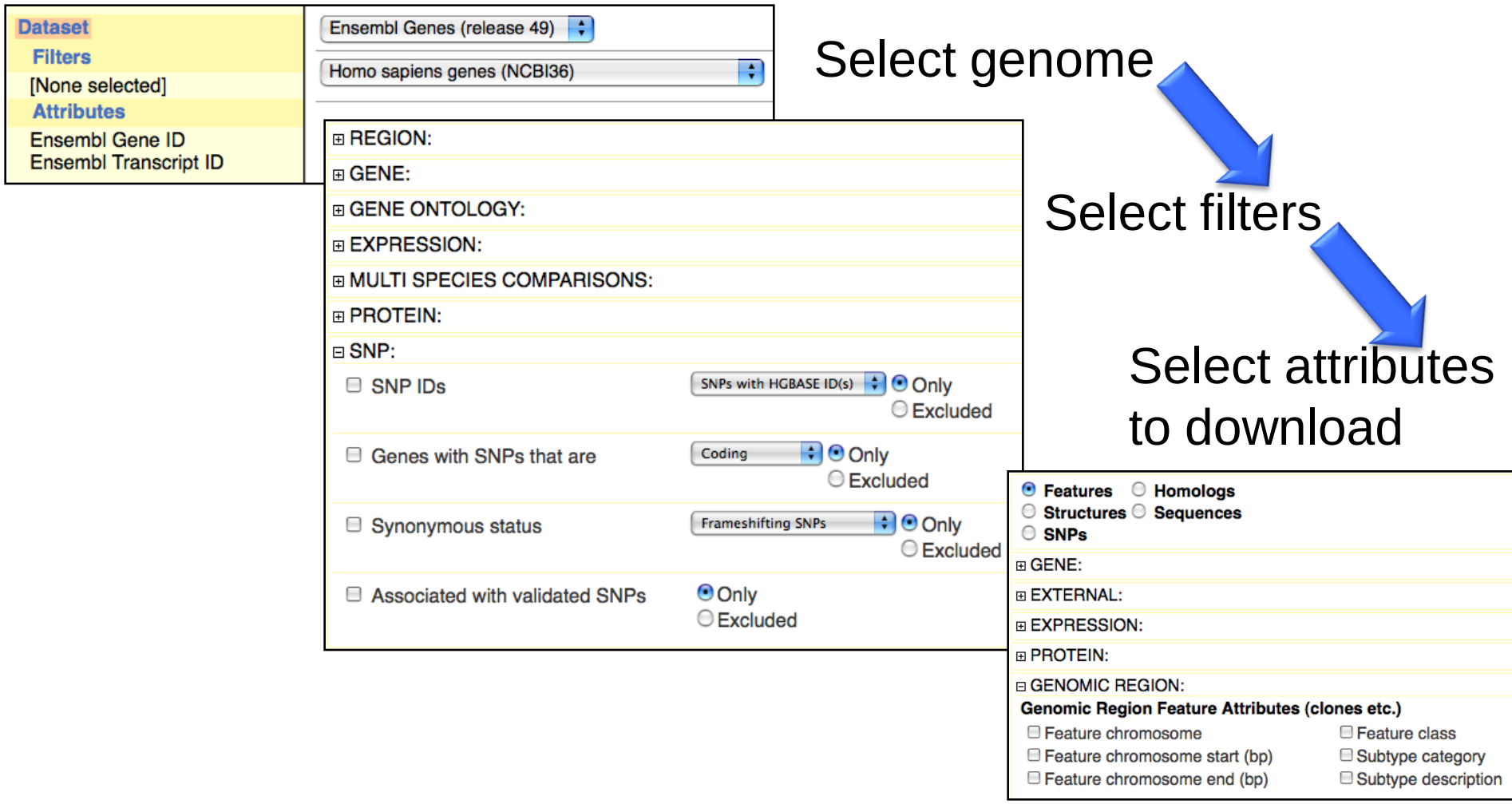
Sources of Gene Attributes

- Ensembl BioMart (eukaryotes)
  - http://www.ensembl.org
- Entrez Gene (general)
  - http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene
- Model organism databases
  - E.g. SGD: http://www.yeastgenome.org/
- Many others: discuss during lab

Ensembl BioMart

- Convenient access to gene list annotation



What Have We Learned?

- Many gene attributes in databases
  - Gene Ontology (GO) provides gene function annotation
    - GO is a classification system and dictionary for biological concepts
    - Annotations are contributed by many groups
    - More than one annotation term allowed per gene
    - Some genomes are annotated more than others
    - Annotation comes from manual and electronic sources
    - GO can be simplified for certain uses (GO Slim)
- Many other gene attributes available from Ensembl and Entrez Gene

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- Interpreting gene lists
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  - BioMart + other sources
- Gene identifiers and mapping

Gene and Protein Identifiers

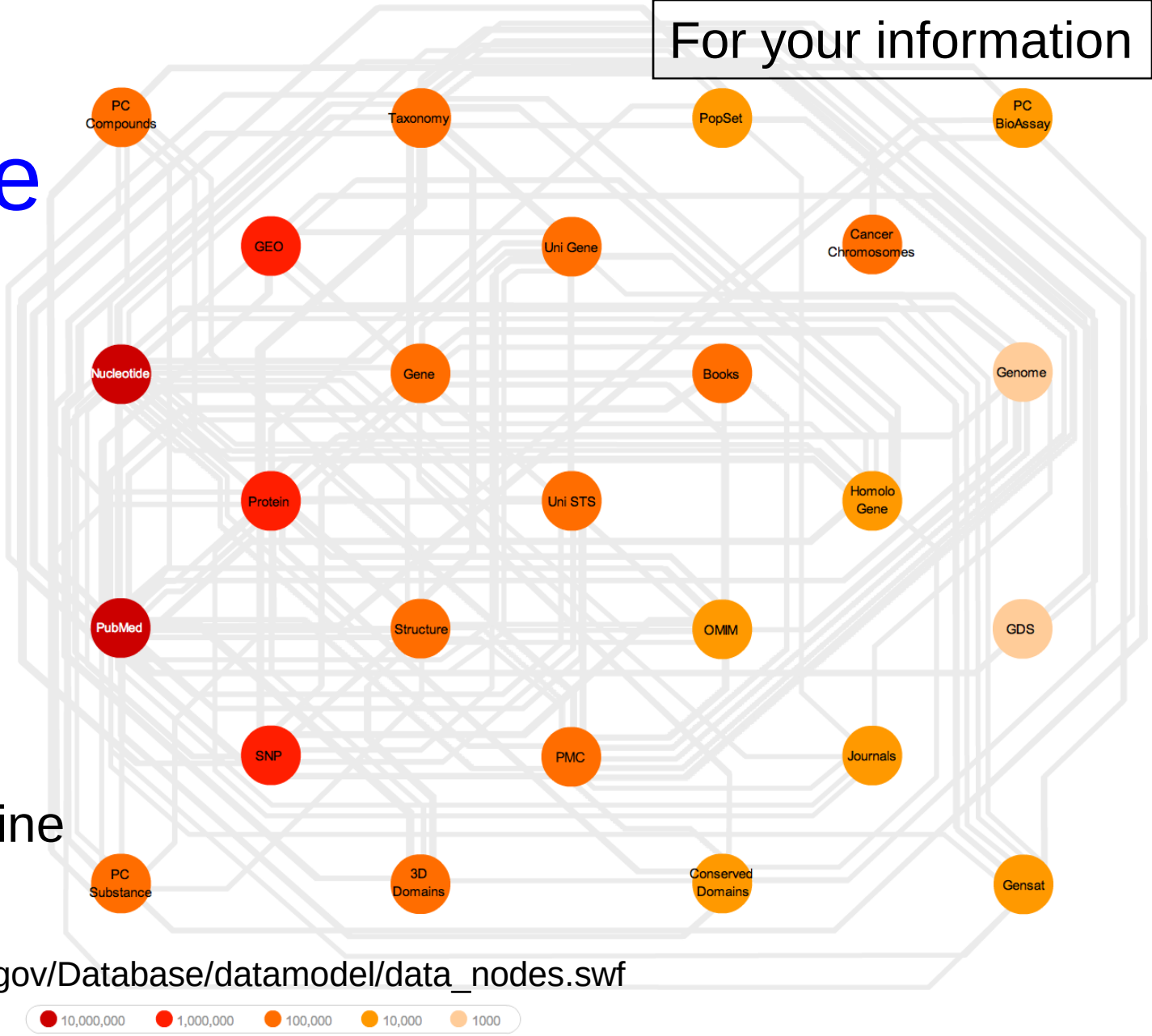
- Identifiers (IDs) are ideally unique, stable names or numbers that help track database records
  - E.g. Social Insurance Number, Entrez Gene ID 41232
- Gene and protein information stored in many databases
  - → Genes have many IDs
- Records for: Gene, DNA, RNA, Protein
  - Important to recognize the correct record type
  - E.g. Entrez Gene records don't store sequence. They link to DNA regions, RNA transcripts and proteins.

For your information

NCBI Database Links

NCBI:
U.S. National Center for Biotechnology Information

Part of National Library of Medicine (NLM)



http://www.ncbi.nlm.nih.gov/Database/datamodel/data_nodes.swf

For your information

Common Identifiers

Gene
Ensembl ENSG00000139618
Entrez Gene 675
Unigene Hs.34012

RNA transcript
GenBank BC026160.1
RefSeq NM_000059
Ensembl ENST00000380152

Protein
Ensembl ENSP00000369497
RefSeq NP_000050.2
UniProt BRCA2_HUMAN or A1YBP1_HUMAN
IPI IPI00412408.1
EMBL AF309413
PDB 1MIU

Species-specific
HUGO HGNC BRCA2
MGI MGI:109337
RGD 2219
ZFIN ZDB-GENE-060510-3
FlyBase CG9097
WormBase WBGene00002299 or ZK1067.1
SGD S000002187 or YDL029W

Annotations
InterPro IPR015252
OMIM 600185
Pfam PF09104
Gene Ontology GO:0000724
SNPs rs28897757

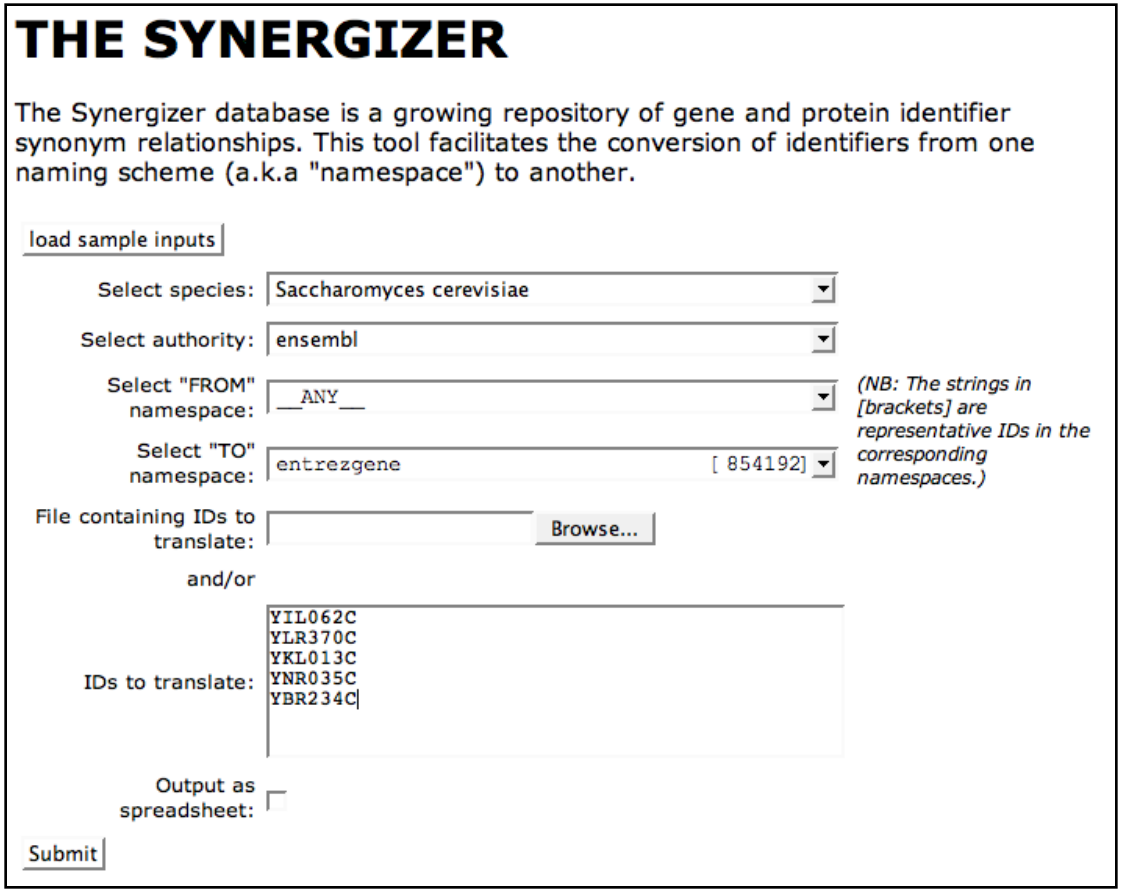
Experimental Platform
Affymetrix 208368_3p_s_at
Agilent A_23_P99452
CodeLink GE60169
Illumina GI_4502450-S

Red = Recommended

Identifier Mapping

- So many IDs!
  - Mapping (conversion) is a headache
- Four main uses
  - Searching for a favorite gene name
  - Link to related resources
  - Identifier translation
    - E.g. Genes to proteins, Entrez Gene to Affy
  - Unification during dataset merging
    - Equivalent records

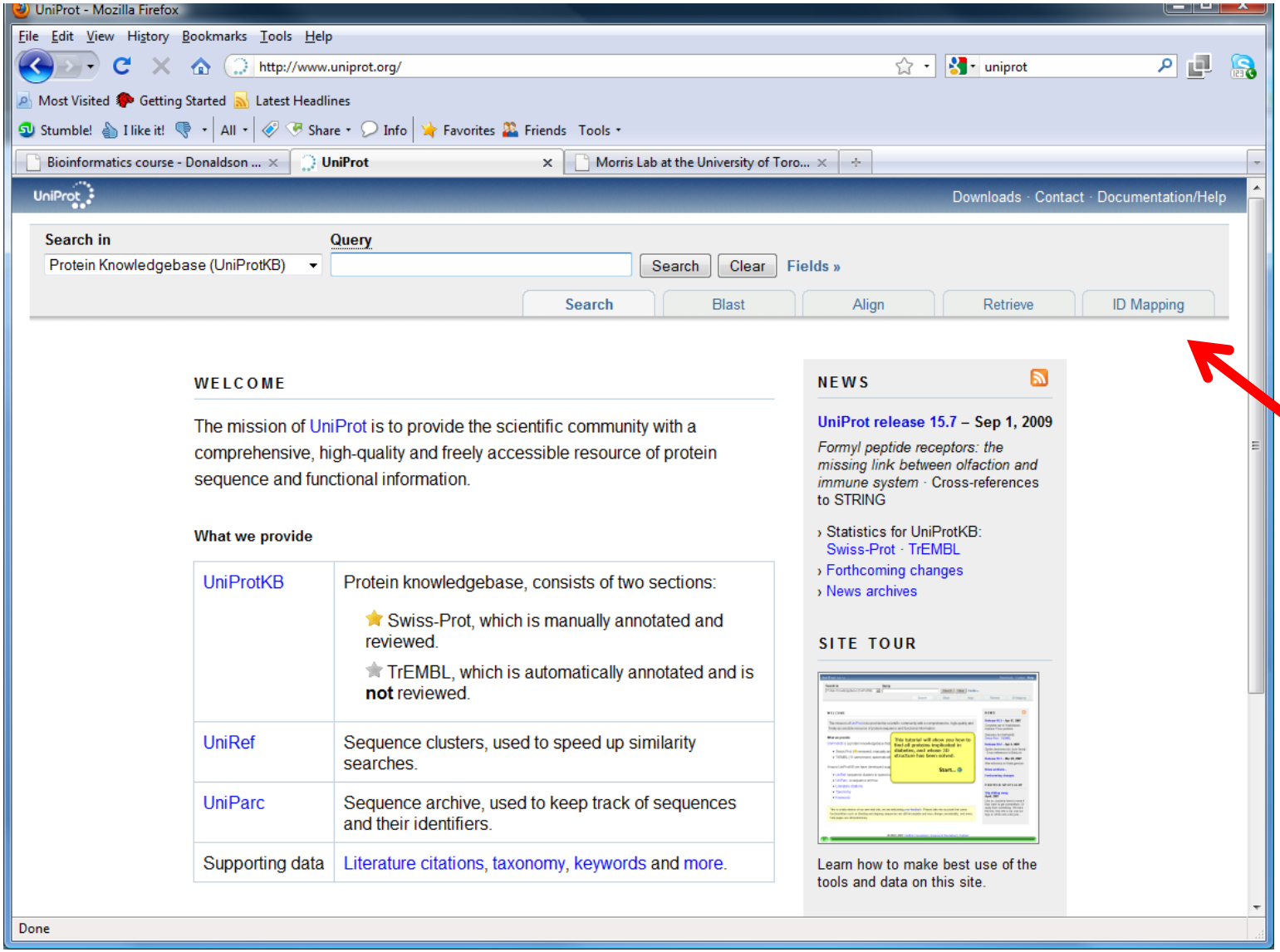
ID Mapping Services



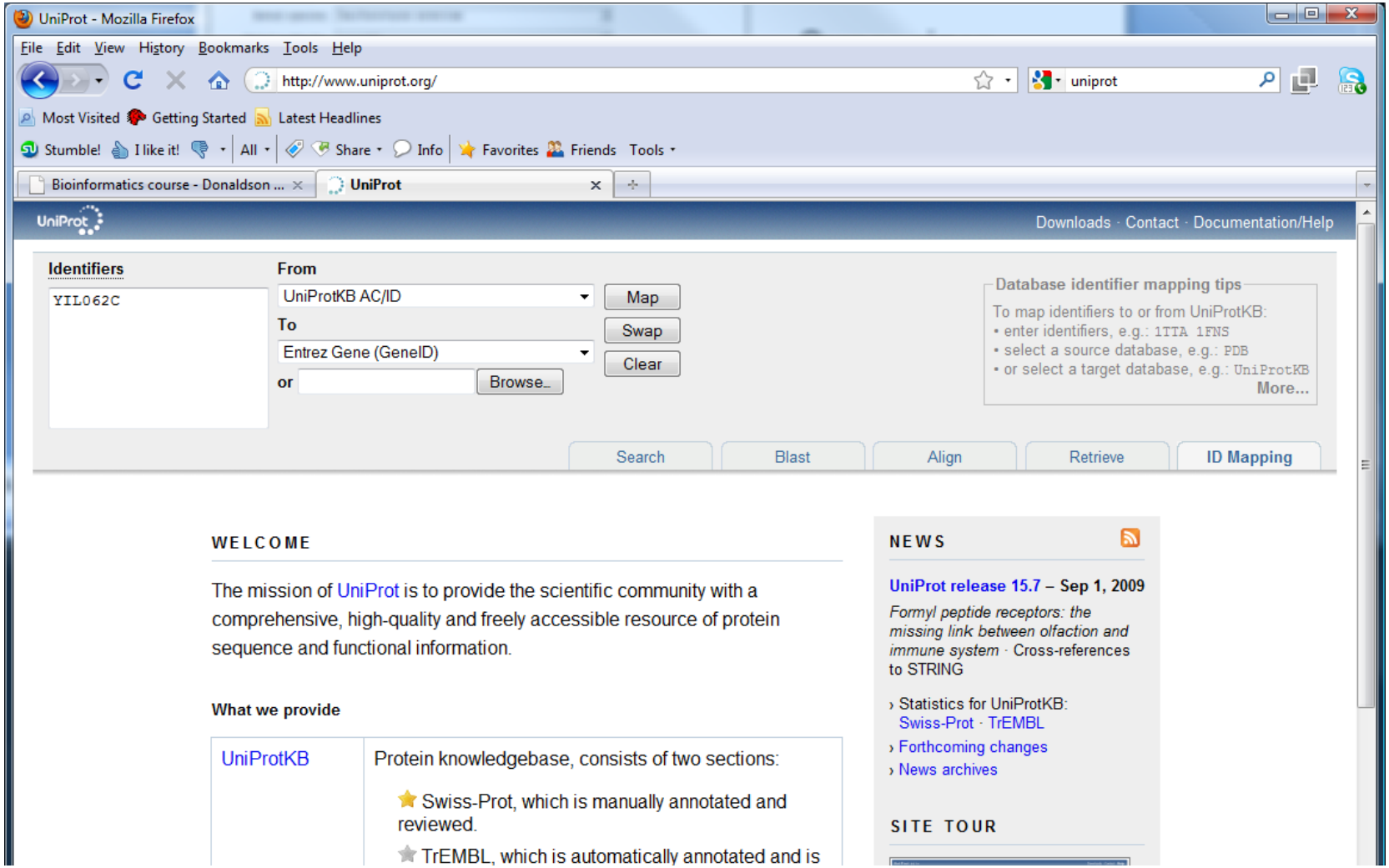
*	entrezgene
YIL062C	854748
YLR370C	851085
YKL013C	853856
YNR035C	855771
YBR234C	852536

- Synergizer
  - http://llama.med.harvard.edu/synergizer/translate/
- Ensembl BioMart
  - http://www.ensembl.org
- UniProt
  - http://www.uniprot.org/

UniProt ID Mapping Service



UniProt ID Mapping Service



ID Mapping Challenges

- Avoid errors: map IDs correctly
- Gene name ambiguity – not a good ID
  - e.g. FLJ92943, LFS1, TRP53, p53
  - Better to use the standard gene symbol: TP53
- Excel error-introduction
  - OCT4 is changed to October-4
- Problems reaching 100% coverage
  - E.g. due to version issues
  - Use multiple sources to increase coverage

Zeeberg BR et al. Mistaken identifiers: gene name errors can be introduced inadvertently when using Excel in bioinformatics BMC Bioinformatics. 2004 Jun 23;5:80

ID Mapping Challenges

- Spot-test any ID mapping service you use.
- Check samples from first, last and middle of your list of identifiers to be converted.
- Ask for help if you are uncertain. This is a field of expertise in itself.

- A new plugin for Cytoscape has been released called CyThesaurus based on bridgedb.org.
- Programmers see also, http://www.bridgedb.org

What Have We Learned?

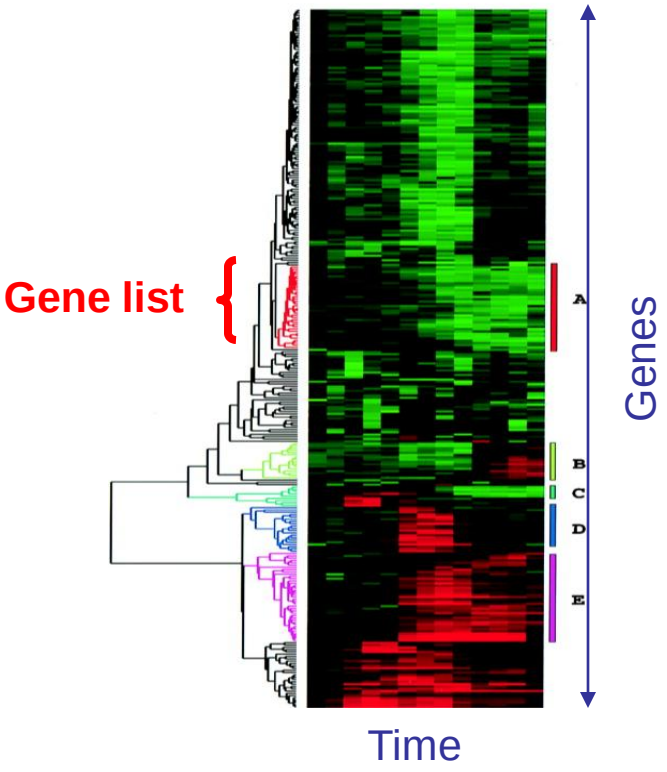
- Genes and their products and attributes have many identifiers (IDs)
- Genomics requirement to convert or map IDs from one type to another
- ID mapping services are available
- Use standard, commonly used IDs to avoid ID mapping challenges
- Spot-test ID mapping services you use

Break

- Over-representation analysis next

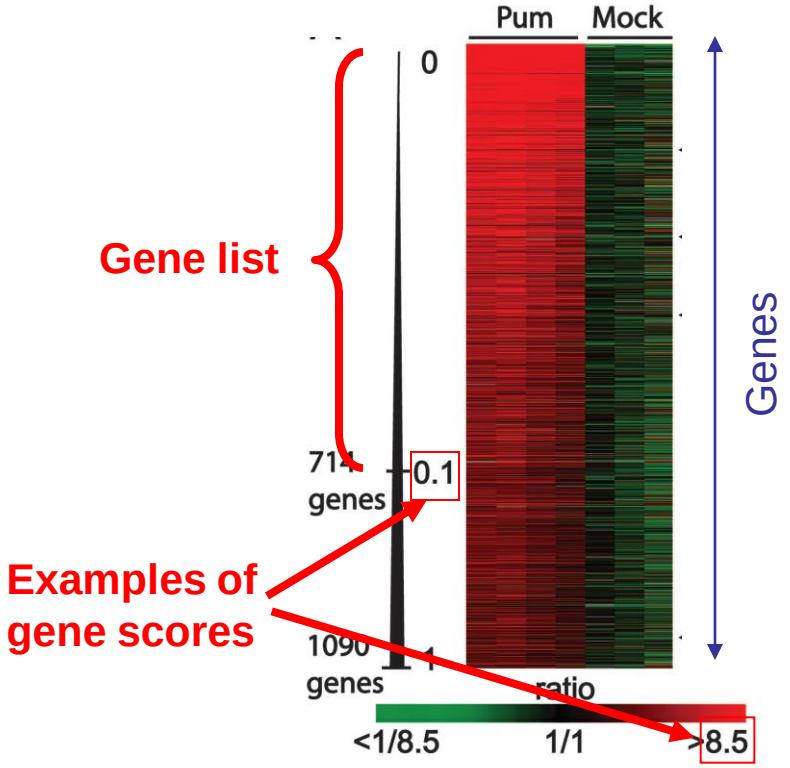
Examples of sources of gene lists

Clustering



Source Eisen et al. (1998) PNAS 95

Thresholding a gene "score"



Source: Gerber et al. (2006) PNAS103

Over-representation analysis (ORA) in a nutshell

- Given:
  1. Gene list: e.g. RRP6, MRD1, RRP7, RRP43, RRP42 (yeast), or Gene Scores: RRP6 (4.0), MRD1 (3.0) etc
  2. Gene annotations: e.g. Gene ontology, transcription factor binding sites in promoter
- ORA Question: *Are any of the gene annotations surprisingly enriched in the gene list?*
- Details:
  - How to assess “surprisingly” (statistics)
  - How to correct for repeating the tests

Overview

- Theory:
  - Review: What is a P-value? The good ole' T-test.
  - Fisher's Exact Test, the bread and butter of ORA
  - Correcting for multiple testing
  - Enrichment analysis with gene rankings

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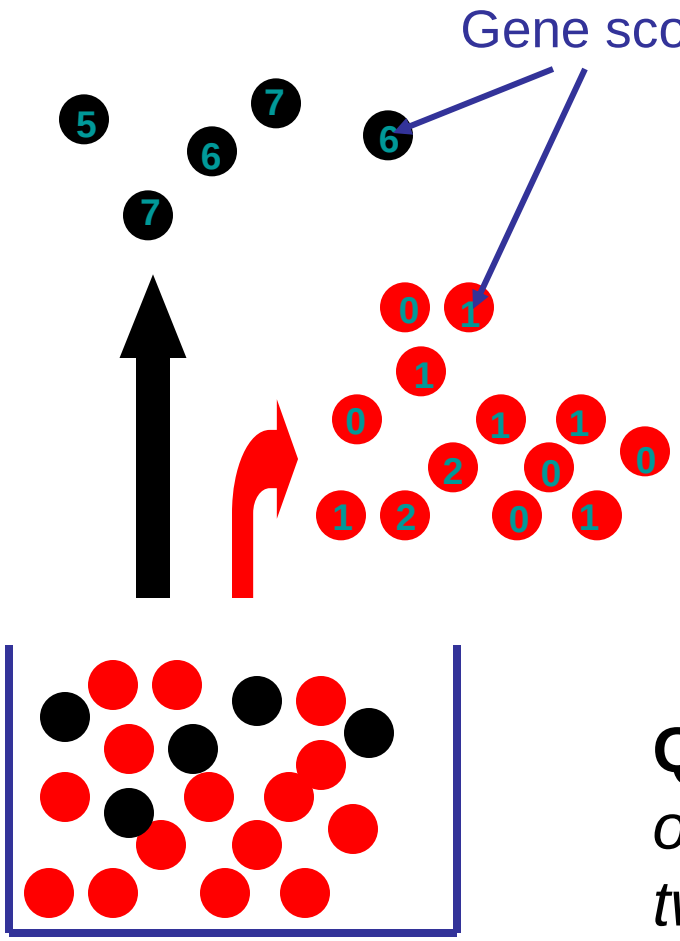
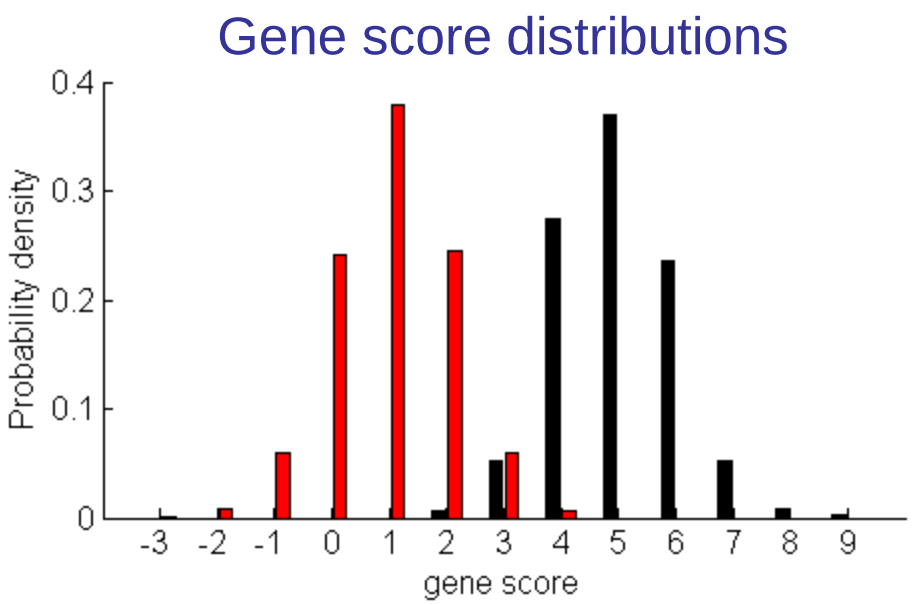
What is a P-value?

- The P-value is (a bound) on the probability that the "null hypothesis" is true,
- Calculated by calculating statistics using the data and testing the probability of observing those statistics, or ones more extreme, given a sample of the same size distributed according to the null hypothesis,
- Intuitively: *P-value is the probability of a false positive result* (aka "Type I error")

The good old T-test





Question: *How likely are the observed differences between the two distributions due to chance?*

ORA using the T-test

Answer: *Two-tailed T-test*

Black: N_1=500

Mean: m_1 = 1.1

Std: s_1 = 0.9

Red: N_2=4500

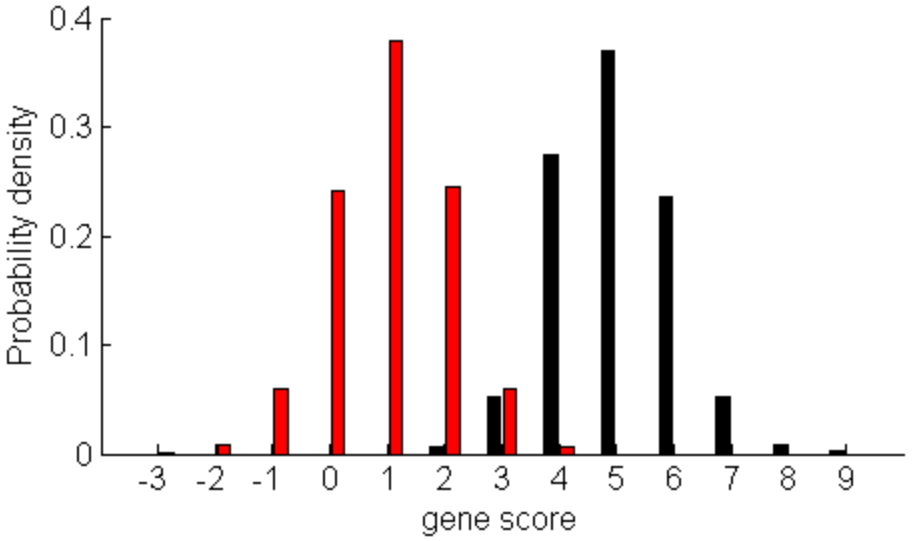
Mean: m_1 = 4.9

Std: s_1 = 1.0

T-statistic = $$\frac{m_1 - m_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

= -88.5

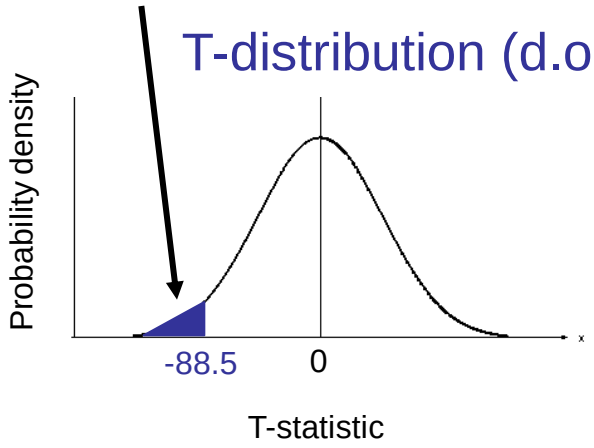
Gene score distributions



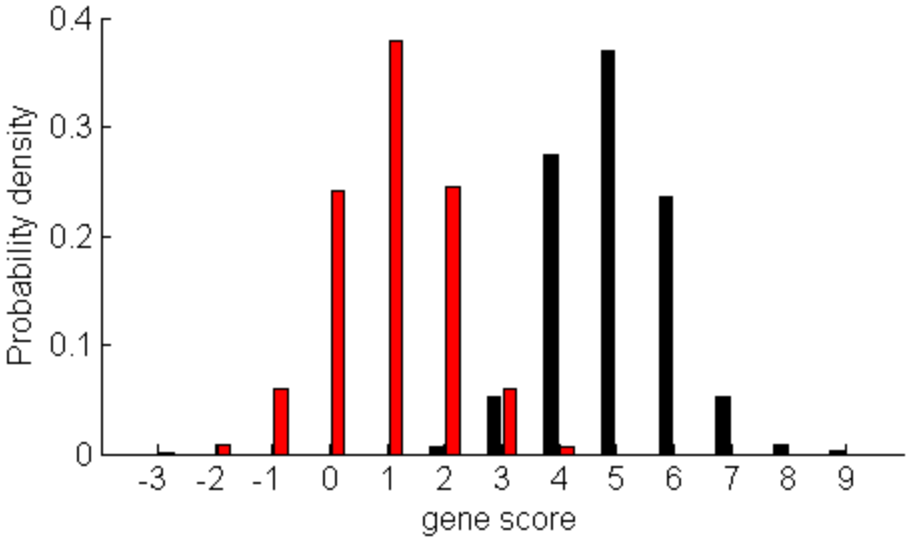
Formal Question: *What is the probability of observing the T-statistic or one more extreme if the means of the two distributions were the same?*

ORA using the T-test

P-value = shaded area * 2



Gene score distributions



$$\text{T-statistic} = \frac{m_1 - m_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

= -88.5

Formal Question: *What is the probability of observing the T-statistic or one more extreme if the means of the two distributions were the same?*

Overview

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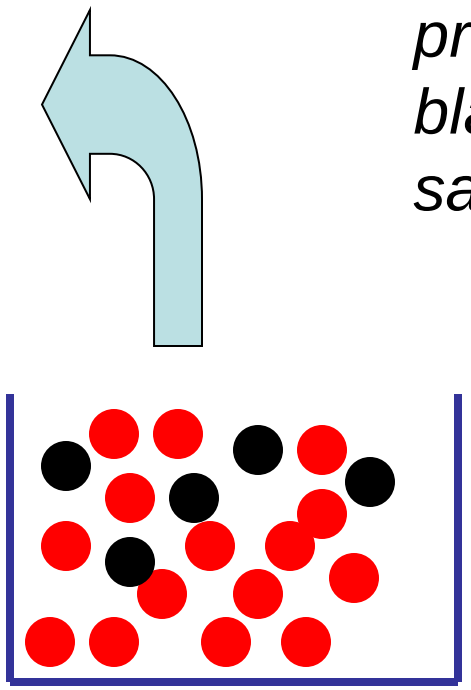
Fisher's exact test: the bread and butter of ORA

a.k.a., the hypergeometric test

Gene list

- RRP6
- MRD1
- RRP7
- RRP43
- RRP42



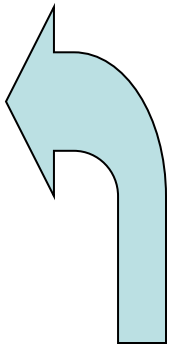
Formal question: *What is the probability of finding 4 or more black genes in a random sample of 5 genes?*

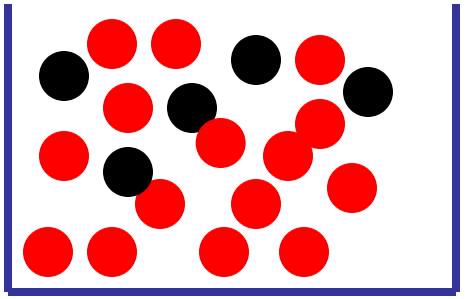
Background population:
500 black genes,
5000 red genes

Fisher's exact test cont.

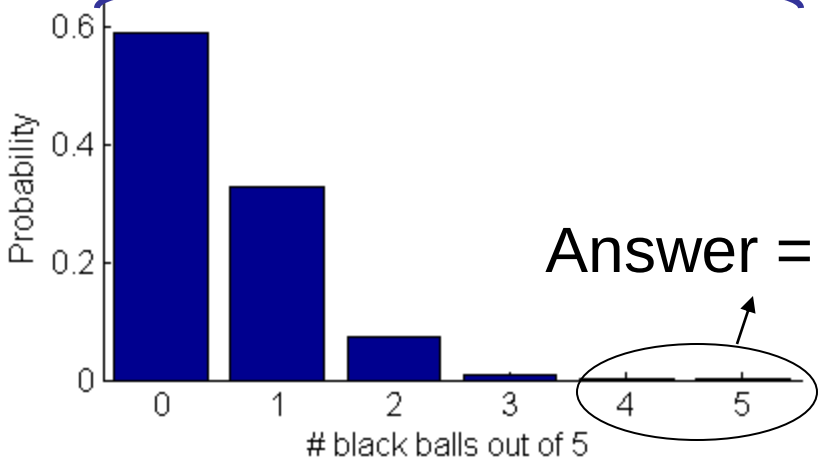
Gene list

- RRP6
- MRD1
- RRP7
- RRP43
- RRP42





Null distribution



P-value

Answer = 4.6×10^{-4}

Background population:

500 black genes,

5000 red genes

Important details

- To test for *under-enrichment* of “black”, test for *over-enrichment* of “red”.
- Need to choose “background population” appropriately, e.g., if only portion of the total gene complement is queried (or available for annotation), only use that population as background.
- To test for enrichment of more than one independent types of annotation (red vs black and circle vs square), apply Fisher’s exact test separately for each type. ***More on this later***

What have we learned?

- Fisher's exact test is used for ORA of gene lists for a single type of annotation,
- P-value for Fisher's exact test
  - is "the probability that a random draw of the same size as the gene list from the background population would produce the observed number of annotations in the gene list or more.",
  - and depends on size of both gene list and background population as well and # of black genes in gene list and background.

Overview

- Theory:
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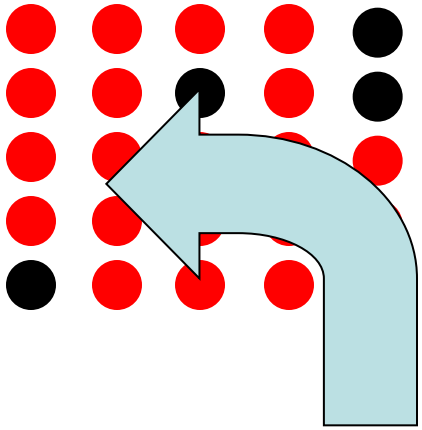
  - Correcting for multiple testing
  - Enrichment analysis with gene rankings

Correcting for multiple testing: overview

- Why do we need to correct? Winning the P-value lottery.
- Controlling the Family-wise Error Rate (FWER) with the Bonferroni-correction
- Controlling the false-discovery rate (FDR): Benjamini-Hochberg, Storey-Tibshirani, Q-values and all that

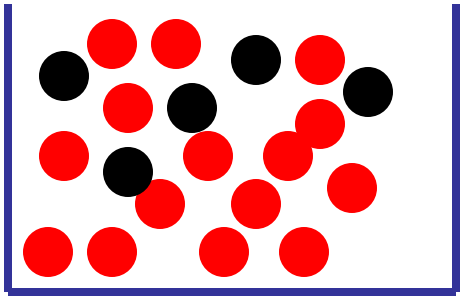
How to win the P-value lottery, part 1

Random draws



… 7,834 draws later …

Expect a random draw with observed enrichment once every 1 / P-value draws

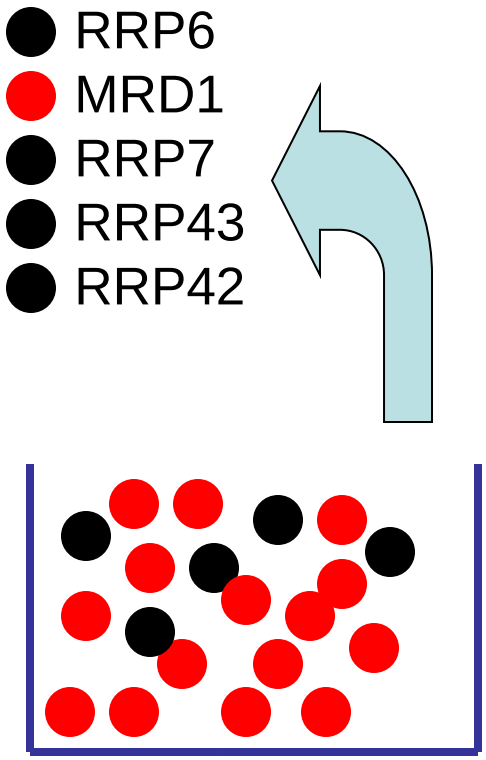


Background population:
500 black genes,
5000 red genes

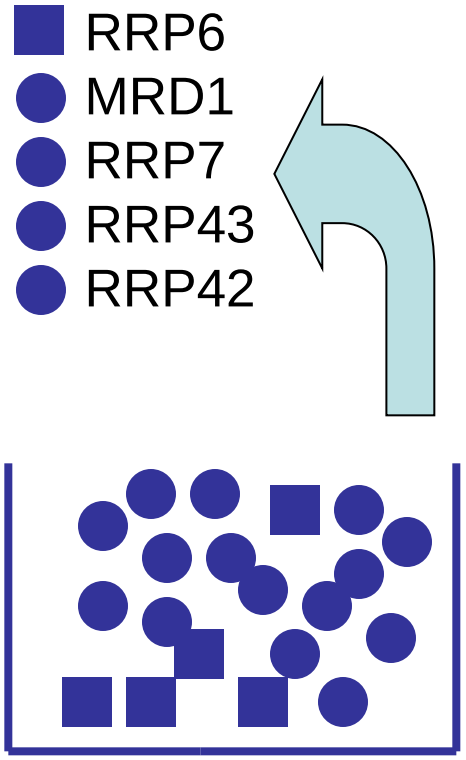
How to win the P-value lottery, part 2

Keep the gene list the same, evaluate different annotations

Observed draw



Different annotations



ORA tests need correction

From the Gene Ontology website:

Current ontology statistics: **25206** terms

- **14825** biological process
- **2101** cellular component
- **8280** molecular function

Two types of multiple test corrections

- Controlling the Family-Wise Error Rate (FWER) controls the probability that any test is a false positive
- Controlling the False Discovery Rate (FDR) controls the proportion of positive tests (i.e. rejections of the null hypothesis) that are false positives

Controlling Family-Wise Error Rate using the Bonferroni correction

If *M* = # of annotations tested:

Corrected P-value = *M* x original P-value

Corrected P-value is greater than or equal to the probability that any single one of the observed enrichments could be due to random draws. The jargon for this correction is **"controlling for the *Family-Wise Error Rate (FWER)*"**

Bonferroni correction caveats

- Bonferroni correction is very stringent and can “wash away” real enrichments.
- Often users are willing to accept a less stringent condition, the “false discovery rate” (FDR), which leads to a gentler correction when there are real enrichments.

False discovery rate (FDR)

- FDR is *the expected **proportion** of the observed enrichments that are due to random chance.*

- Compare to Bonferroni correction which is *the probability that **any one** of the observed enrichments is due to random chance.*

Benjamini-Hochberg (B-H) FDR

If α is the desired FDR (ie level of significance), then choose the corresponding cutoff for the original P-values as follows:

1) Rank all "M" P-values

2) Test each P-value against
$q = \alpha$ x (M-Rank+1) / M
e.g. Let *M = 100, $\alpha = 0.05$*

P-value	Rank	**q**	Is P-value < q?
0.9	1	0.05 X 1.00	No
0.7	2	0.05 x 0.99	No
0.5	3	0.05 X 0.98	No
0.04	4	0.05 x 0.97	Yes
…	…	...	…
0.005	M	0.05 x 0.01	No

3) New P-value cutoff, i.e. "α", is lowest ranked P-value to pass the test.

P-value cutoff of 0.04 ensures FDR < 0.05

Reducing multiple test correction stringency

- The correction to the P-value threshold α depends on the # of tests that you do, so, no matter what, the more tests you do, the more sensitive the test needs to be
- Can control the stringency by reducing the number of tests: e.g. use GO slim or restrict testing to the appropriate GO annotations.

What have we learned

- When testing multiple annotations, need to correct the P-values (or, equivalently, α) to avoid winning the P-value lottery.
- There are two types of corrections:
  - **Bonferroni** controls the probability any one test is due to random chance (aka FWER) and is very stringent
  - **B-H** controls the FDR, i.e., expected proportion of "hits" that are due to random chance
- Can control stringency by carefully choosing which annotation categories to test.

DAVID, part 1

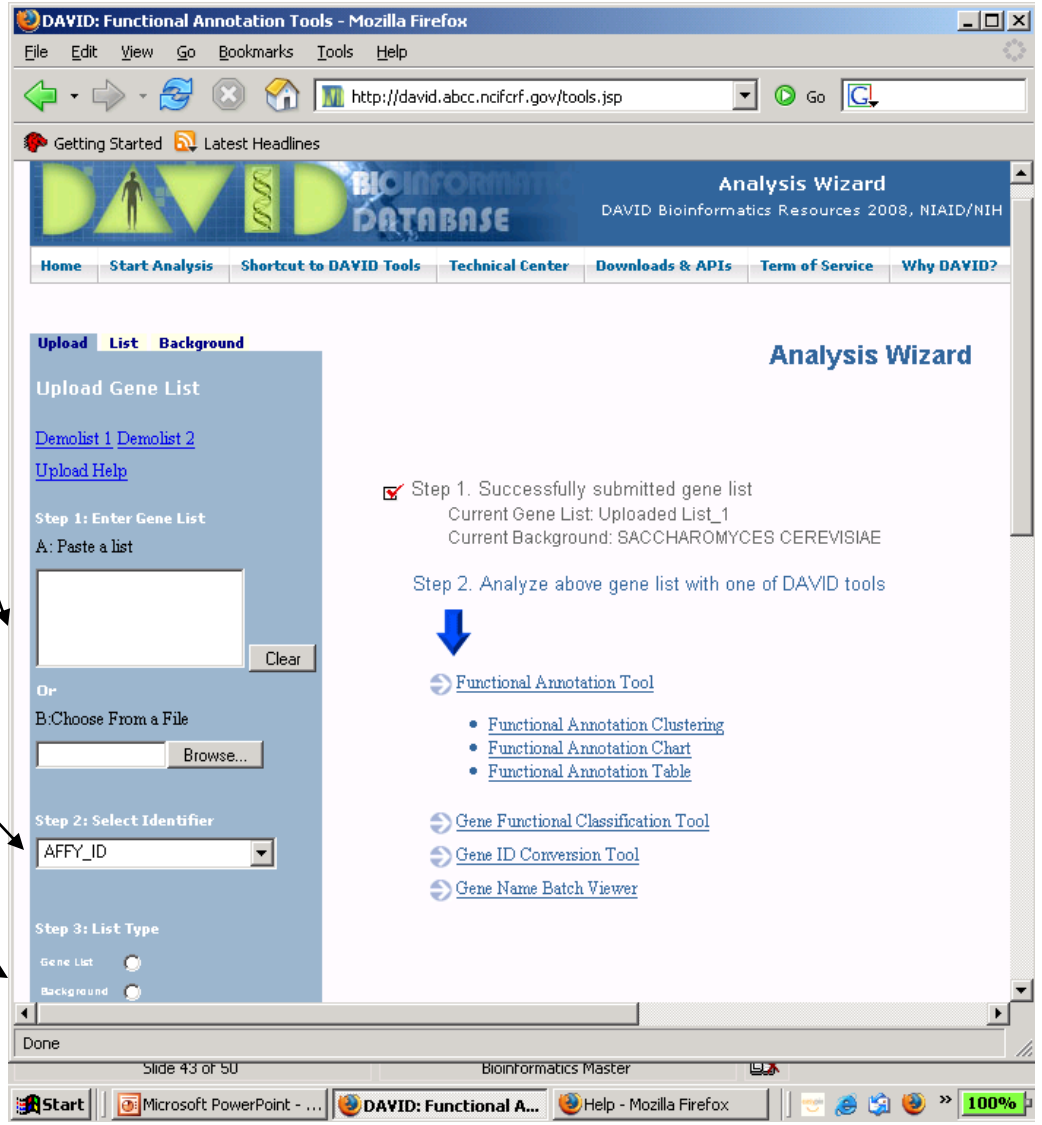
http://david.abcc.ncifcrf.gov/

Paste list here

DAVID automatically detects organism

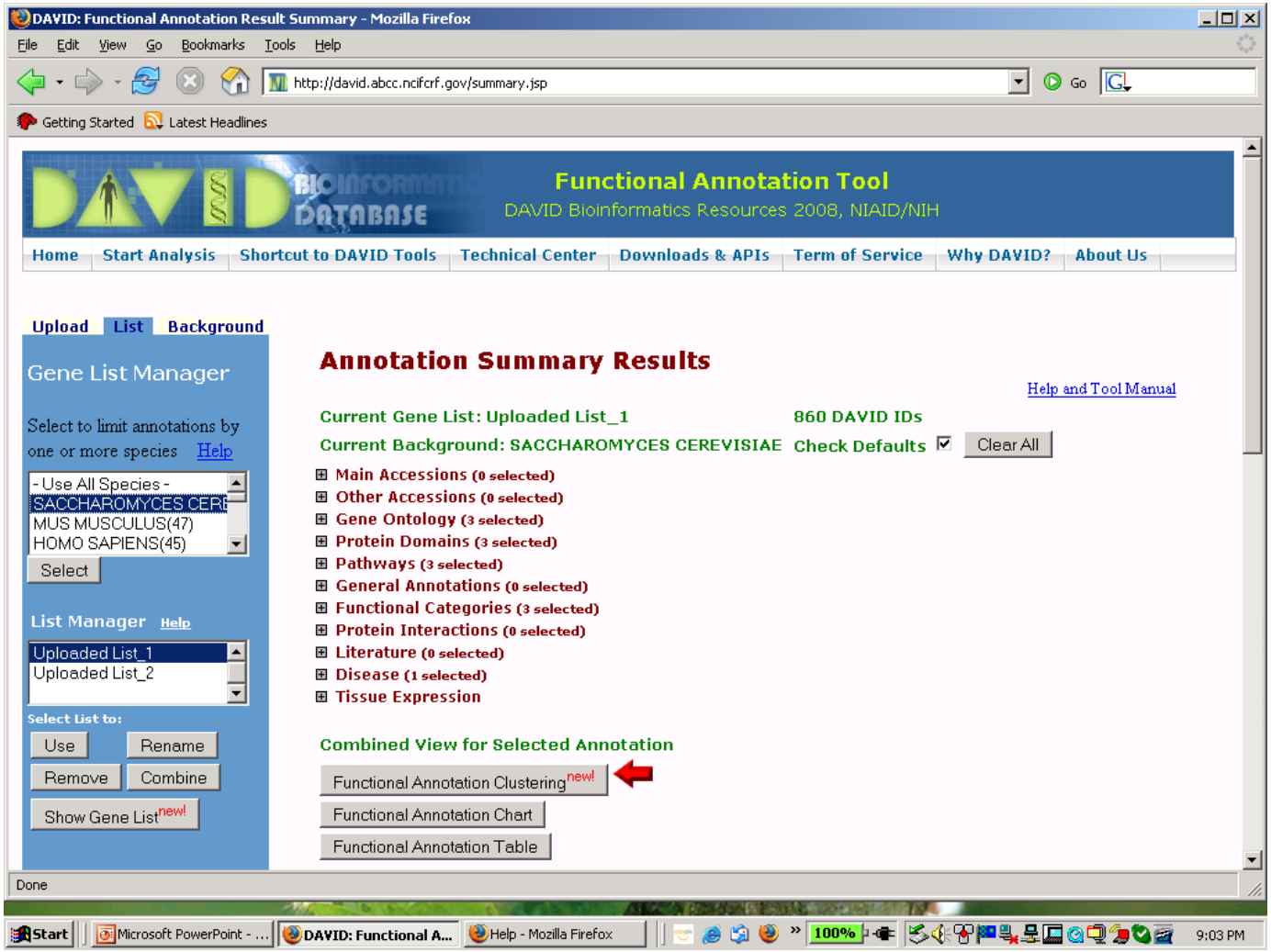
Choose ID type

List type: list or background?



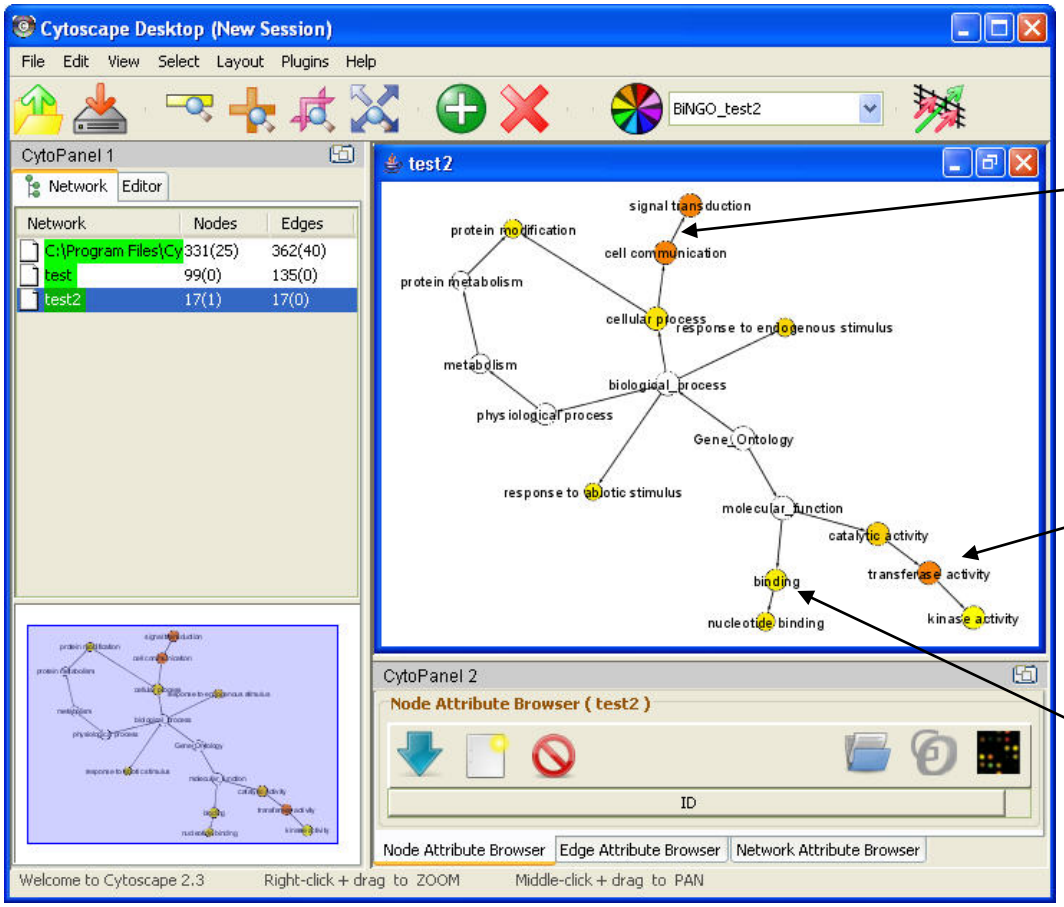
DAVID, part 2

http://david.abcc.ncifcrf.gov/



BINGO, an ORA cytoscape plugin

http://www.psb.ugent.be/cbd/papers/BiNGO/index.htm



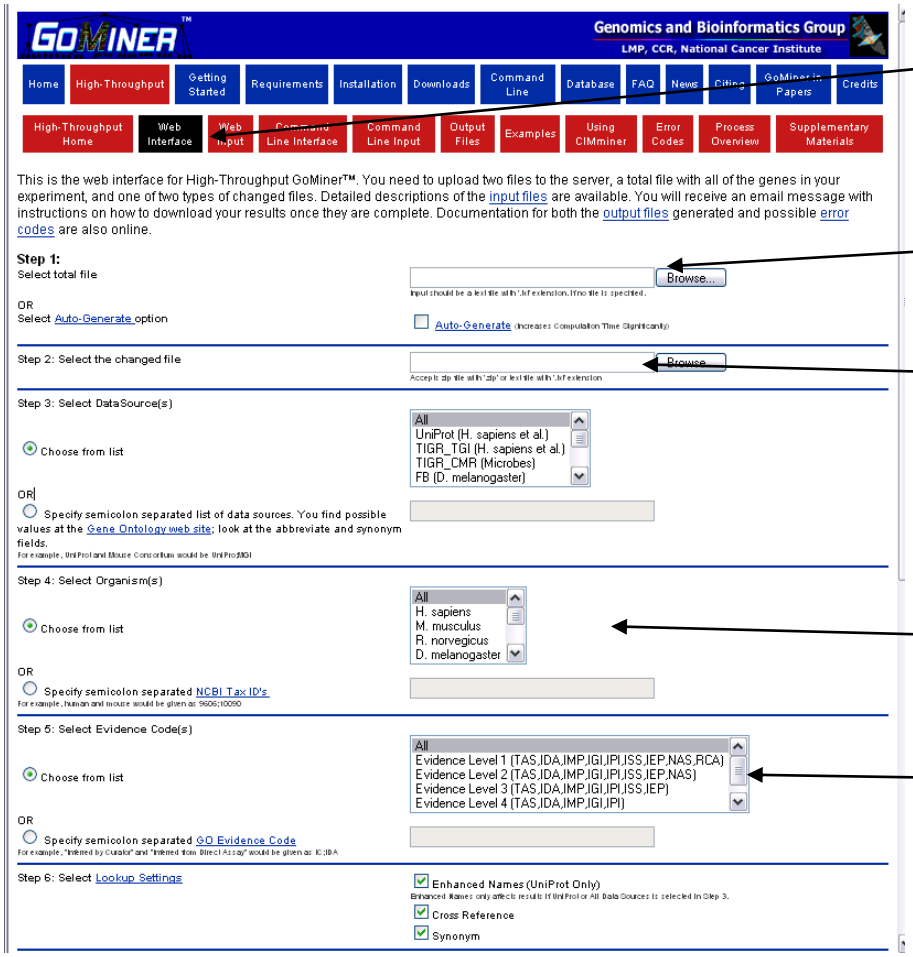
Links represent parent-child relationships in GO ontology

Colours represent significance of enrichment

Nodes represent GO categories

GoMiner, part 1

http://discover.nci.nih.gov/gominer



1. Click “web interface”

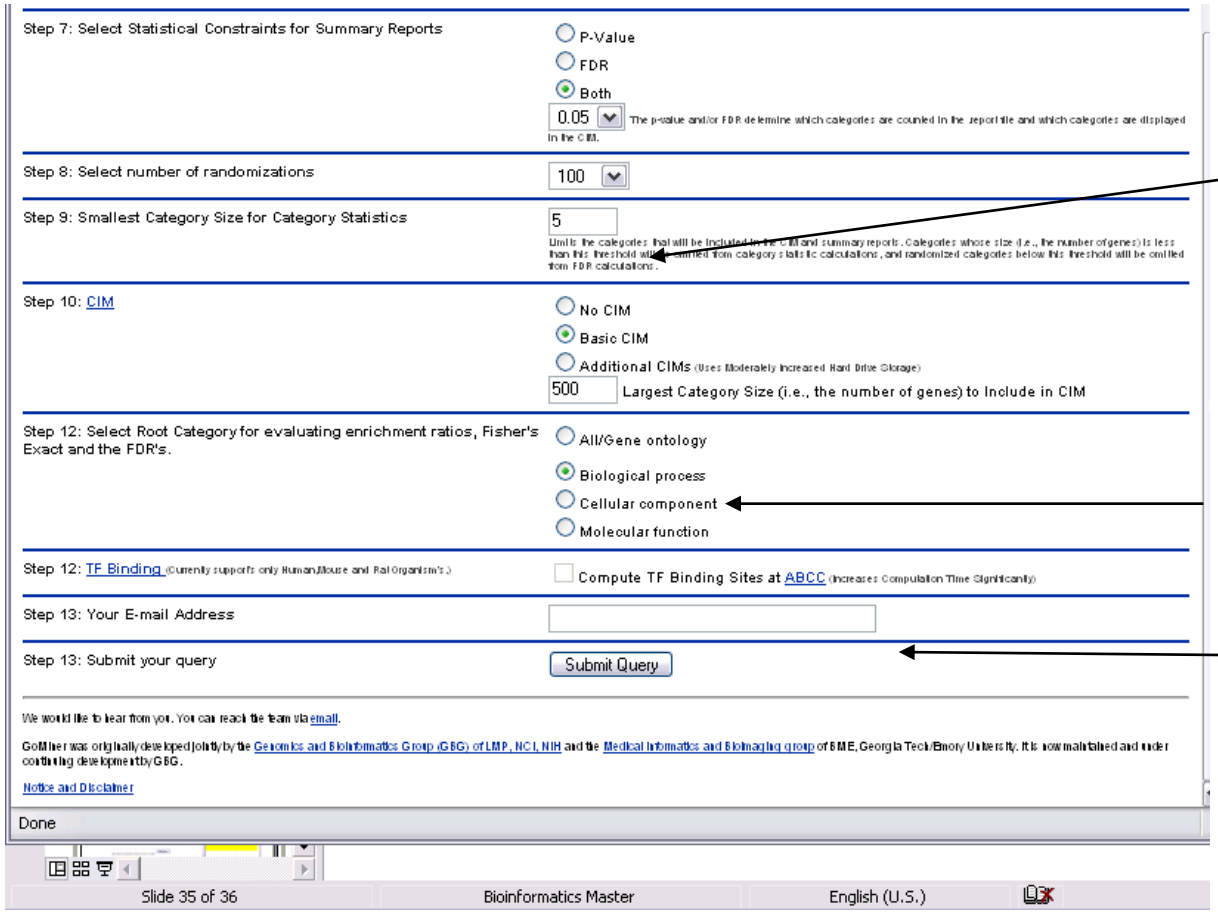
2. Upload names of background genes

3. Upload gene list

4. Choose organism

5. Choose evidence code (All or Level 1)

GoMiner, part 2



6. Restrict # of tests via category size

7. Restrict # of tests via GO hierarchy

8. Results emailed to this address, in a few minutes

Break

- Try out an over-representation analysis with multiple test correction using DAVID.

Overview

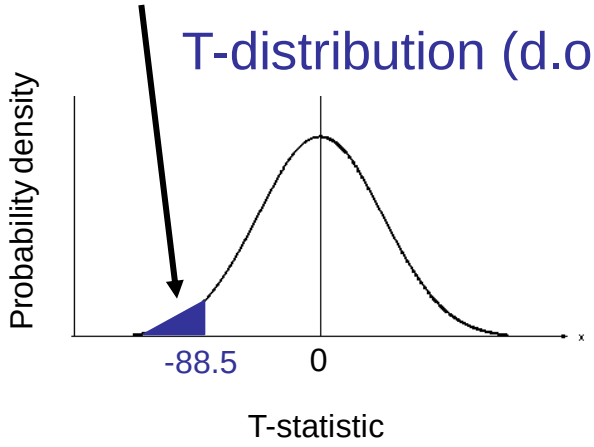
- Theory:
  - Review: What is a P-value? The good ole' T-test.
  - Fisher's Exact Test, the bread and butter of ORA
  - Correcting for multiple testing
  - Enrichment analysis with gene rankings

ORA with gene rankings: overview

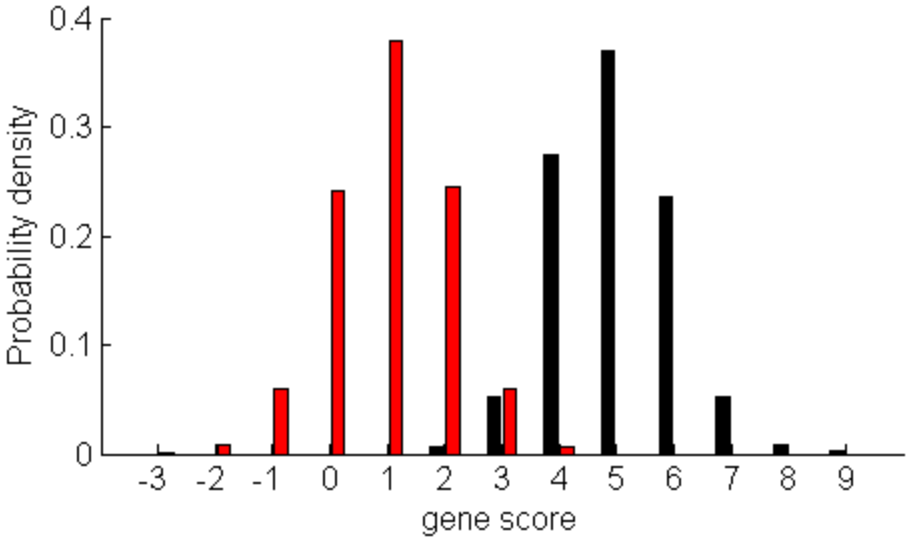
- Why can't I use the T-test?
- The Wilcoxon-Mann-Whitney (WMW) test: a T-test on ranks
- The Kolmogorov-Smirnov (KS) test: testing for arbitrary differences between gene score distributions.
- GSEA

Reminder: ORA using the T-test

P-value = shaded area * 2



Gene score distributions



$$\text{T-statistic} = \frac{m_1 - m_2}{\sqrt{\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}}}$$

= -88.5

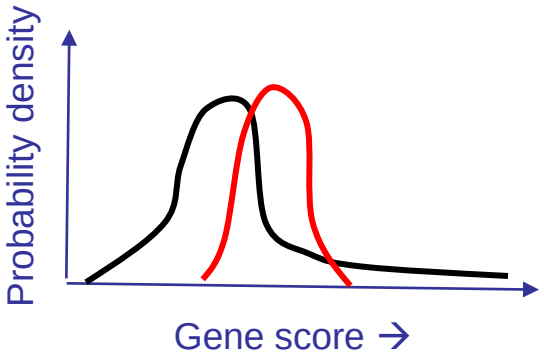
Formal Question: *What is the probability of observing the T-statistic or one more extreme if the means of the two distributions were the same?*

Why can’t we use the T-test?

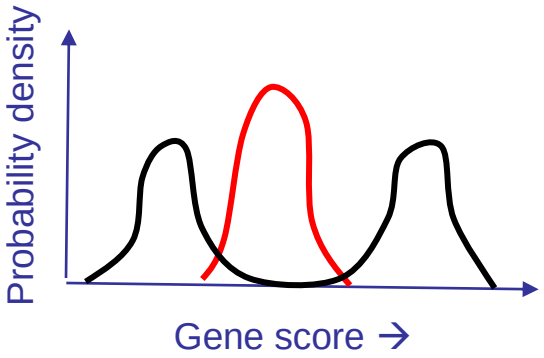
1. Assumes black and red gene score distributions are both approximately Gaussian (i.e. normal)
   - Score distribution assumption is often true for:
     - Log ratios from microarrays
   - Score distribution assumption is rarely true for:
     - Peptide counts, sequence tags (SAGE or NextGen sequencing), transcription factor binding sites hits
2. Tests for significance of difference in means of two distribution but does not test for other differences between distributions.

Examples of inappropriate score distributions for T-tests

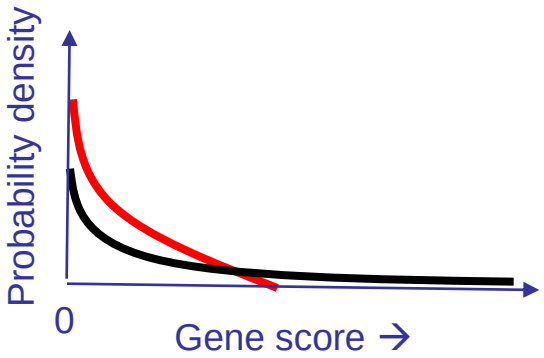
Distributions with gene score outliers, or “heavy-tailed” distributions



Bimodal “two-bumped” distributions.



Gene scores are positive and have increasing density near zero, e.g. sequence counts



Solutions:

1) Robust test for difference of medians (WMW)
2) Direct test of difference of distributions (K-S)

Wilcoxon-Mann-Whitney (WMW) test

aka Mann-Whitney U-test, Wilcoxon rank-sum test

1) Rank gene scores, calculate R_B, sum of ranks of black gene scores

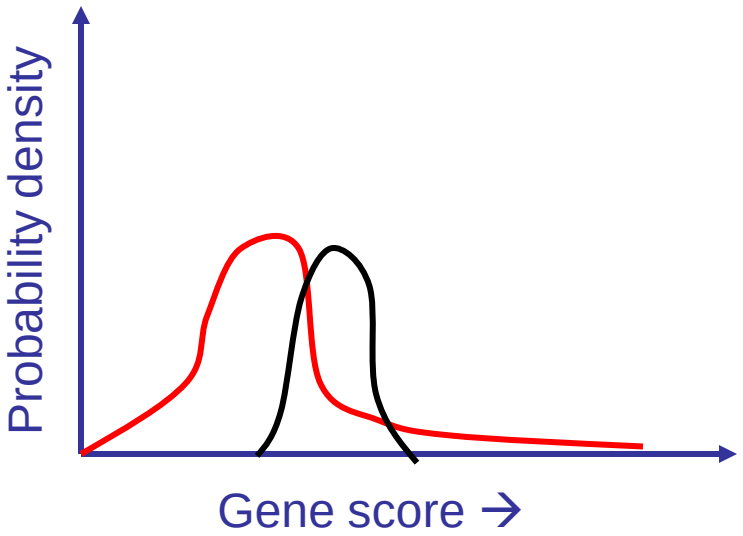
		ranks
2.1	6.5	1
5.6	5.6	2
-1.1	4.5	3
-2.5	3.2	4
-0.5	2.1	5
	1.7	6
	0.1	7
	-1.1	8
	-2.5	9
	-0.5	10

N_2 red gene scores

3.2
1.7
6.5
4.5
0.1

N_1 black gene scores

$R_B = 21$



Formal Question: *Are the medians of the two distributions significantly different?*

Wilcoxon-Mann-Whitney (WMW) test

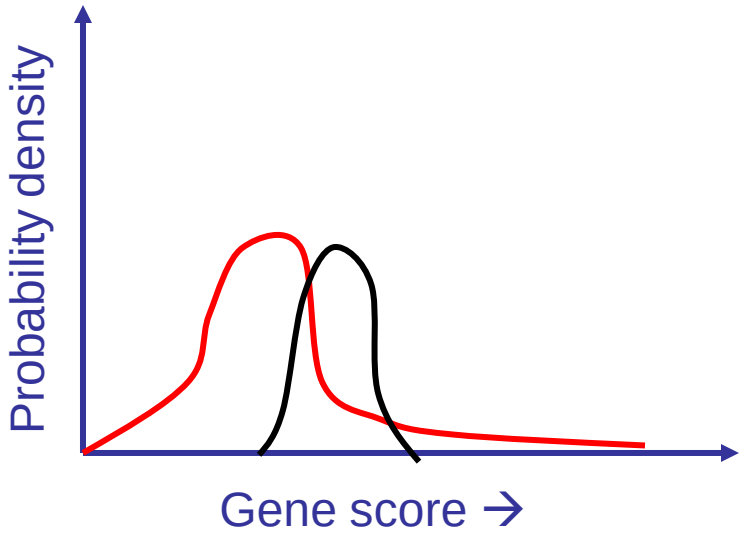
aka Mann-Whitney U-test, Wilcoxon rank-sum test

2) Calculate Z-score:

mean rank

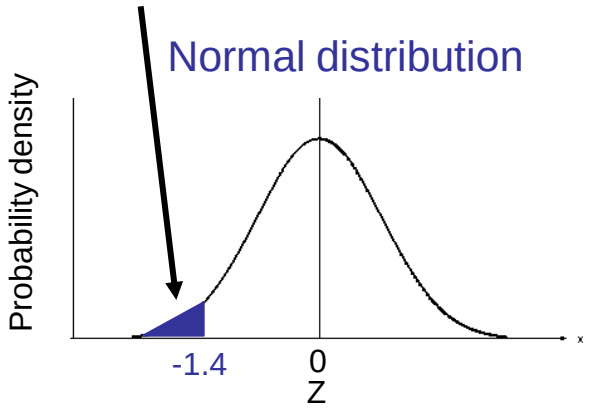
$$Z = \frac{R_B - N_1 \frac{N_1 + N_2 + 1}{2}}{\sigma_U} = -1.4$$

$R_B = 21$



3) Calculate P-value:

P-value = shaded area * 2



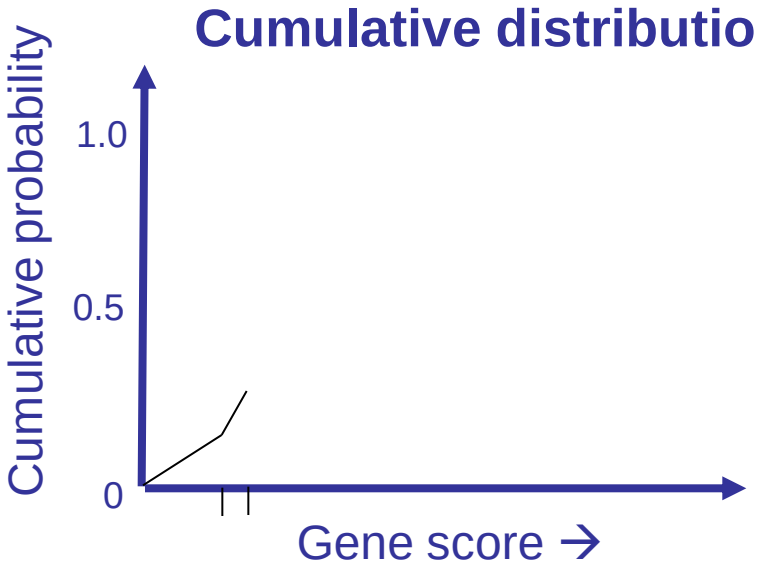
Formal Question: *Are the medians of the two distributions significantly different?*

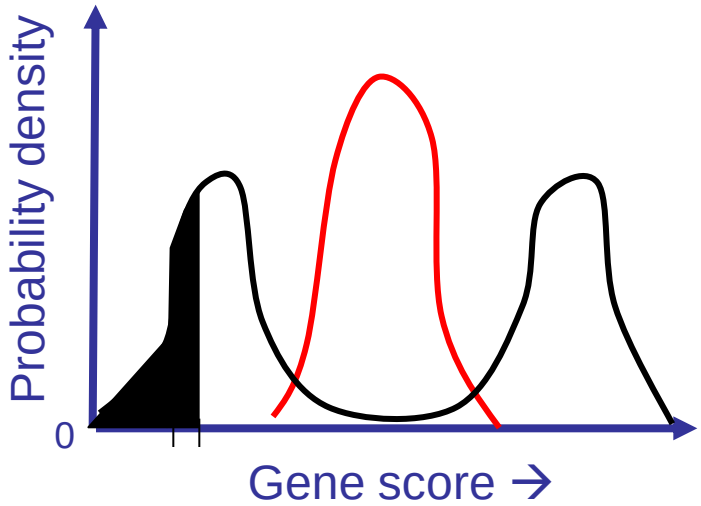
WMW test details

- Described method is only applicable for large N_1 and N_2 and when there are no tied scores, WMW software uses a tied rank correction
- In most cases the WMW test is simply a T-test applied to the ranks of the gene scores
- WMW test is robust to (a few) outliers
-

$$\sigma_u = \sqrt{N_1 N_2 (N_1 + N_2 + 1)/12}$$

Kolmogorov-Smirnov (K-S) test

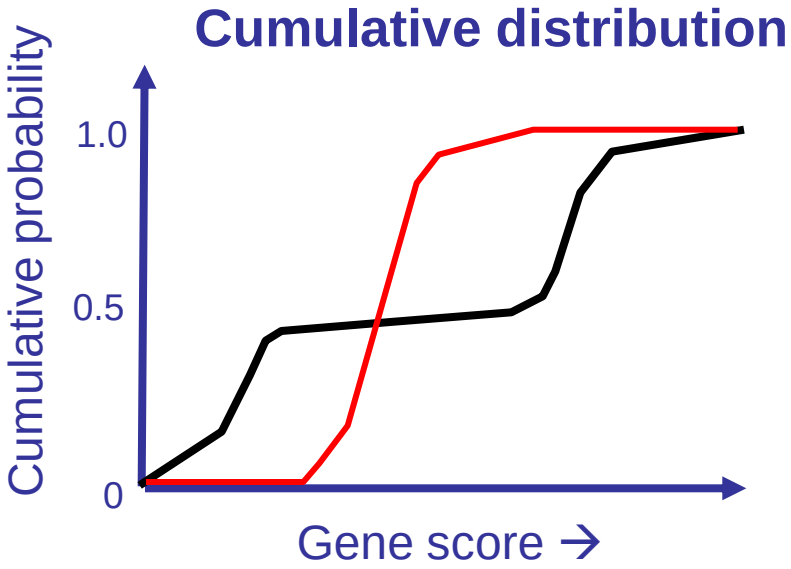


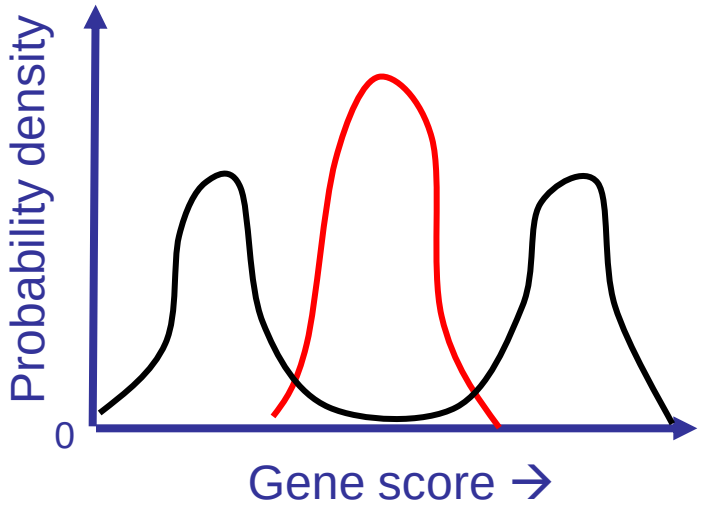


1) Calculate cumulative distributions of red and black

Question: *Are the red and black distributions significantly different?*

Kolmogorov-Smirnov (K-S) test





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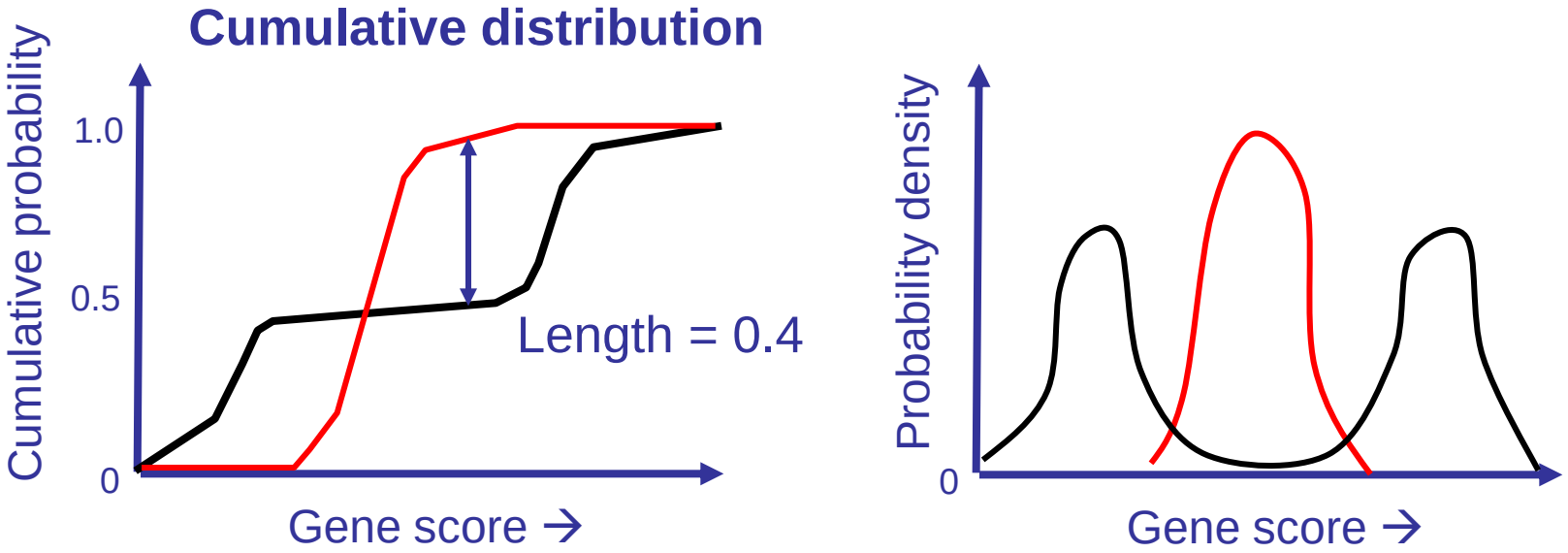


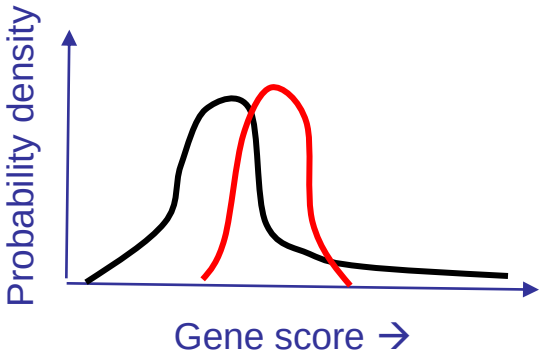
Formal question: *Is the length of largest difference between the "empirical distribution functions" statistically significant?*

WMW and K-S test caveats

- Neither tests is as sensitive as the T-test, ie they require more data points to detect the same amount of difference, so use the T-test whenever it is valid.
- K-S test and WMW can give you different answers: K-S detects difference of distributions, WMW detects difference of medians
- Rare problem: Tied scores and small # of observations can be a problem for some implementations of the WMW test

Proper tests for different distributions

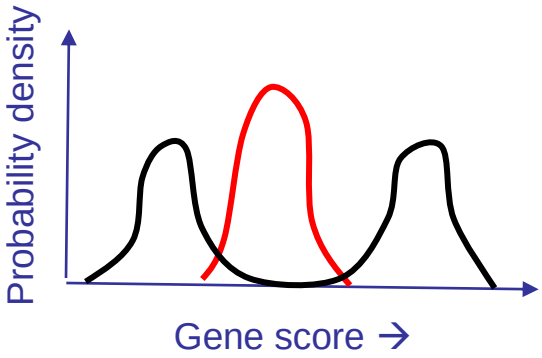
Distributions with gene score outliers, or “heavy-tailed” distributions



Recommended test:

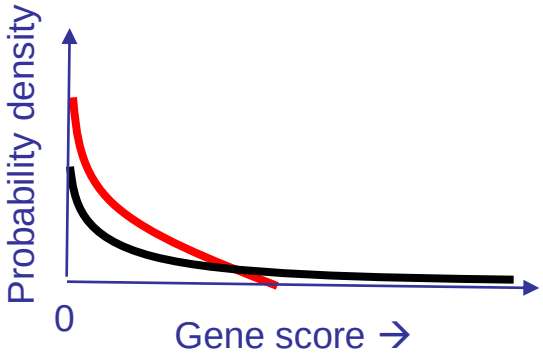
WMW or K-S

Bimodal “two-bumped” distributions.



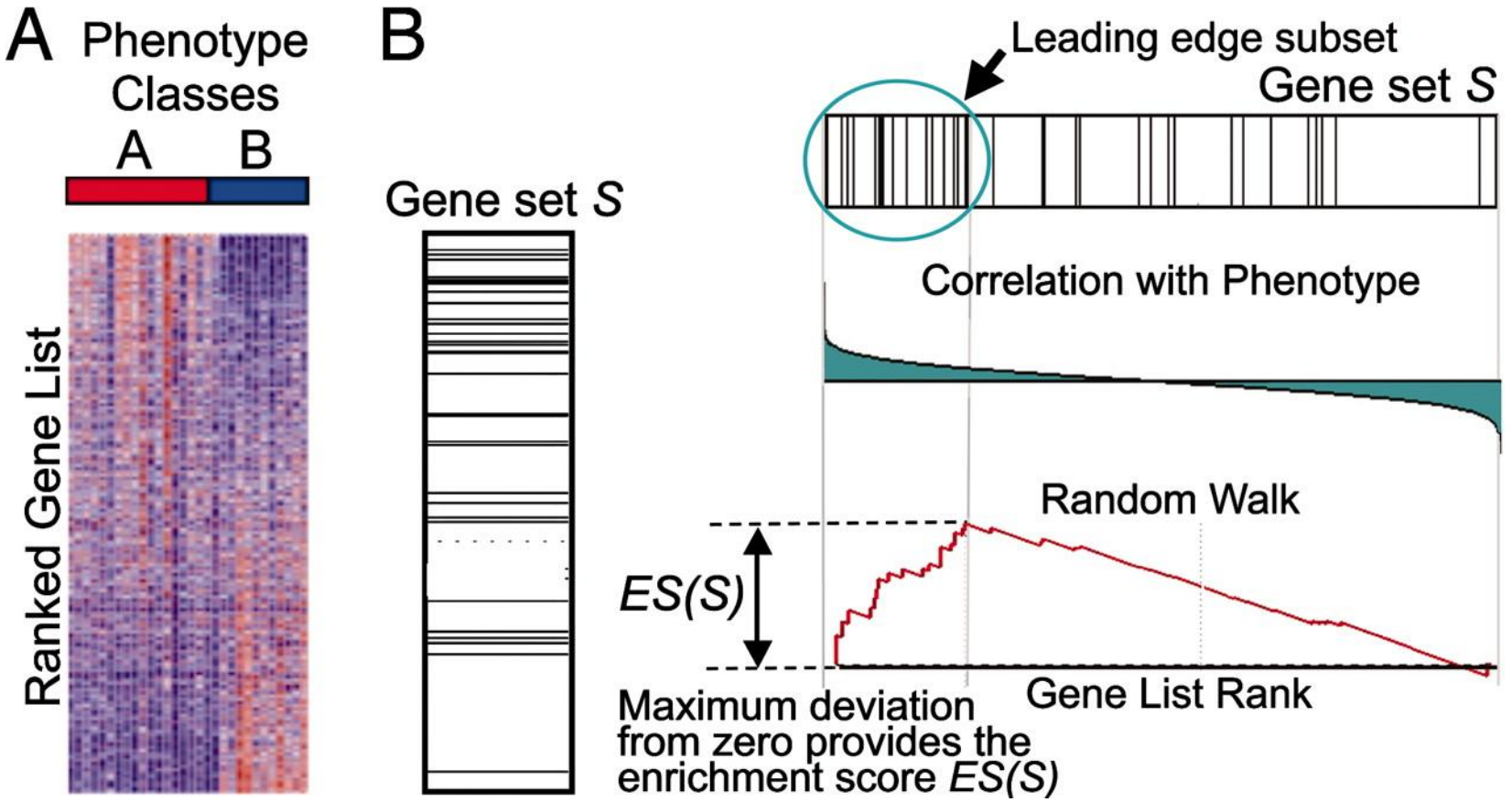
K-S only

Gene scores are positive and have increasing density near zero, e.g. sequence counts



WMW or K-S

A GSEA overview illustrating the method



Subramanian A. et.al. PNAS;2005;102:15545-15550

What have we learned?

- T-test is not valid when one or both of the score distributions is not normal,
- If need a “robust” test, or to test for difference of medians use WMW test or GSEA,
- To test for overall difference between two distributions, use K-S test.

Other common tests and distributions

- Chi-squared (contingency table) test
  - Useful if there are >2 values of annotation (e.g. red genes, black genes, and blue genes)
  - Used as an approximation to Fisher's Exact Test but is inaccurate for small gene lists
- Binomial test
  - Tests if gene scores for red and black either come from either N flips of the same coin or different coins.
  - E.g. black genes are "expressed" in, on average, 5 out of 12 conditions and red genes are expressed in, on average, 2 out of 12 conditions, is the probability of being expressed significantly different for the black and red genes?

Overview

- Theory:
  - Review: What is a P-value? The good ole' T-test.
  - Fisher's Exact Test, the bread and butter of ORA
  - Correcting for multiple testing
  - Enrichment analysis with gene rankings

Break

- Try out an over-representation analysis on gene ranks using GSEA

- GSEA:
  - http://www.broad.mit.edu/gsea/
  - http://www.broadinstitute.org/gsea/downloads.jsp
  - http://www.broadinstitute.org/gsea/doc/desktop_tutorial.jsp
  - http://www.broadinstitute.org/gsea/datasets.jsp

Questions?

Network Visualization/Analysis Introduction

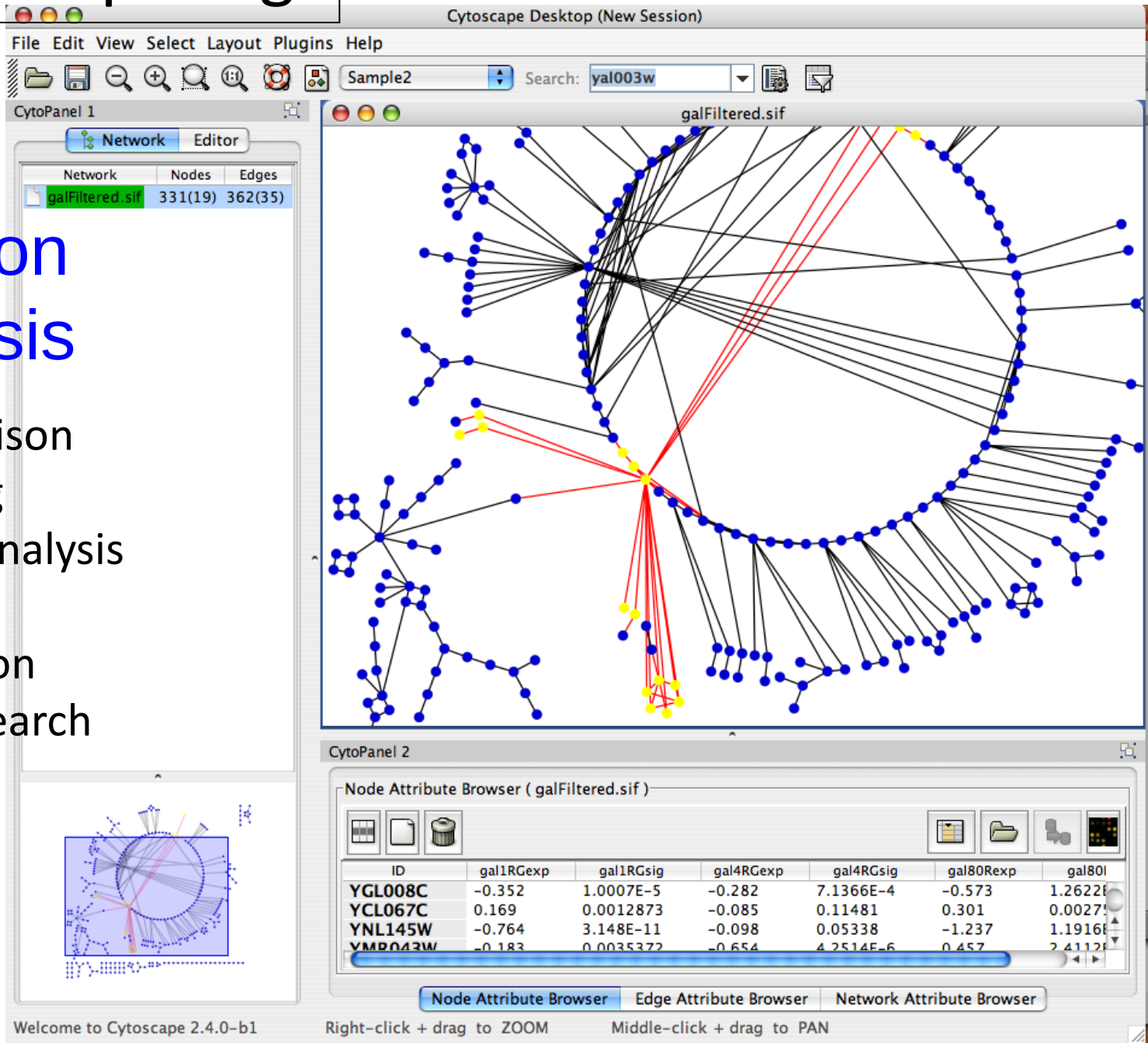
- Network visualization and analysis using Cytoscape software
- Focus on Cytoscape basics now
- Network analysis using Cytoscape in module 4

http://cytoscape.org

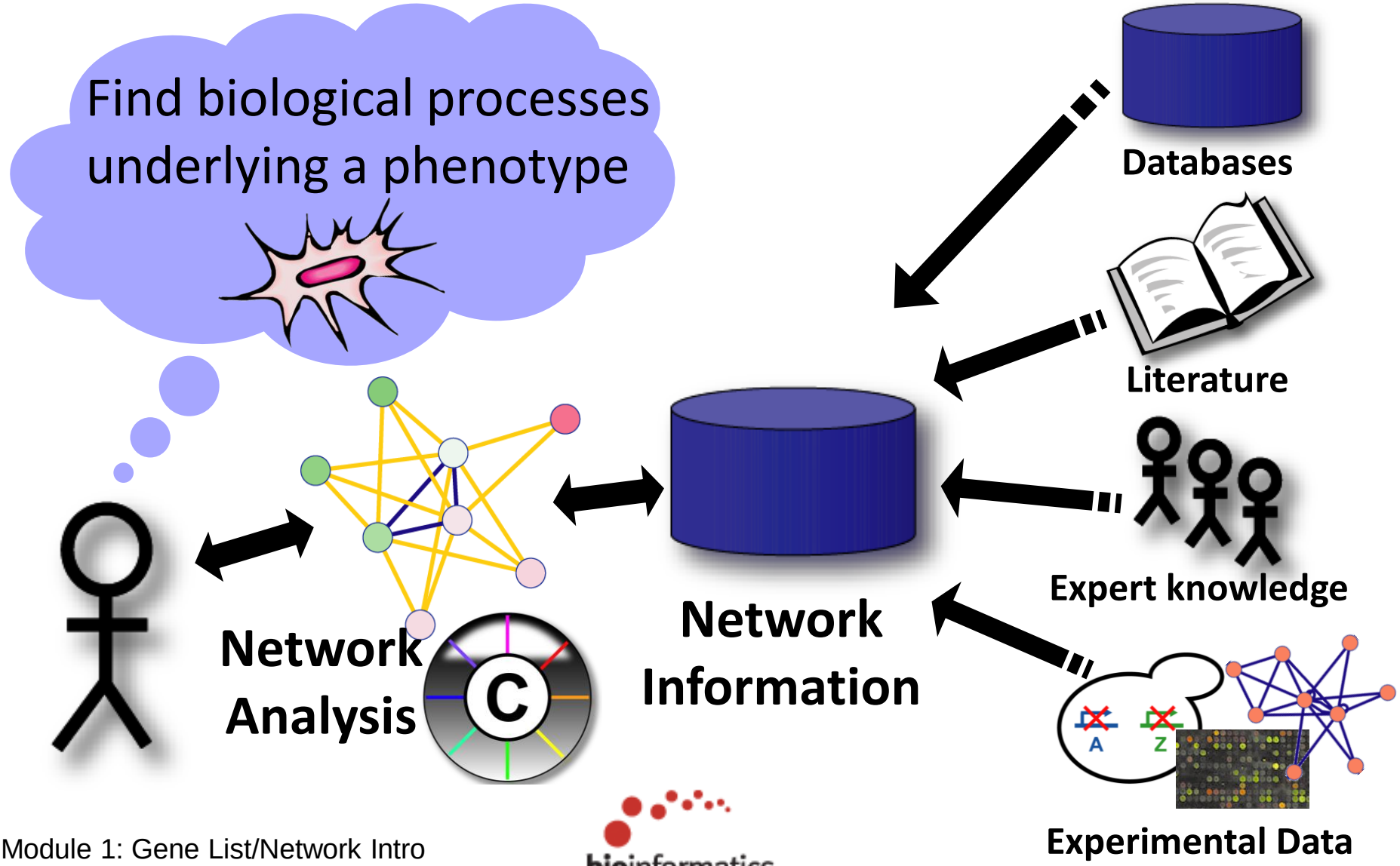
Network visualization and analysis

Pathway comparison
Literature mining
Gene Ontology analysis
Active modules
Complex detection
Network motif search

UCSD, ISB, Agilent, MSKCC, Pasteur, UCSF, Unilever, UToronto, U Texas



Network Analysis using Cytoscape



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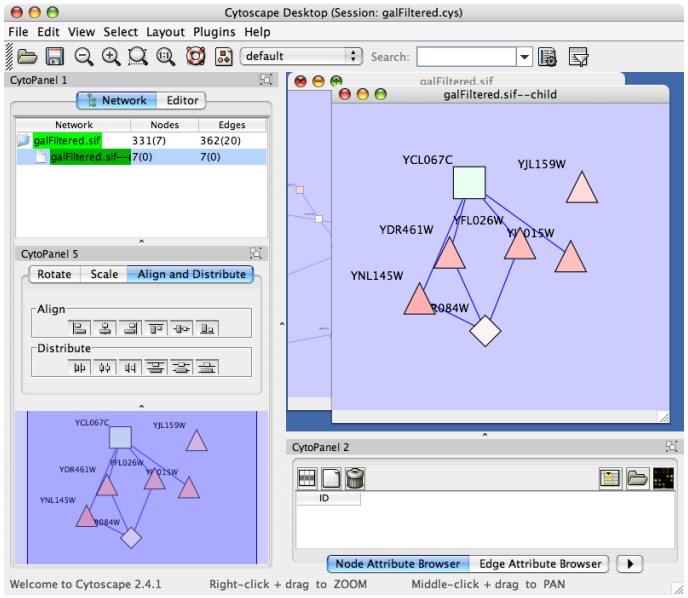
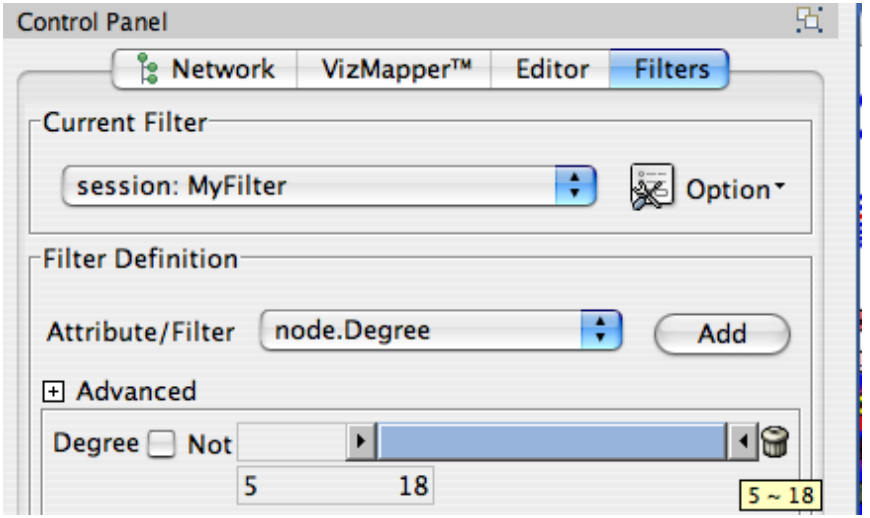
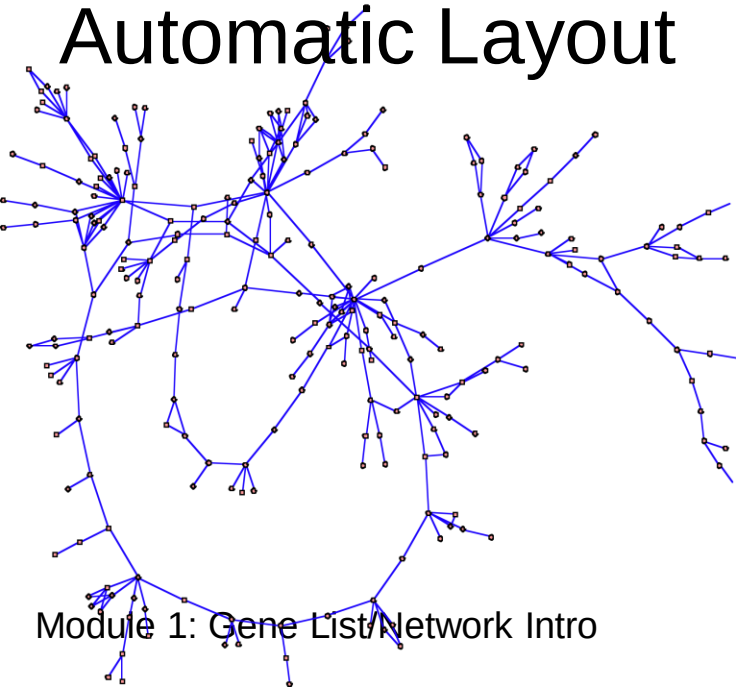
Manipulate Networks



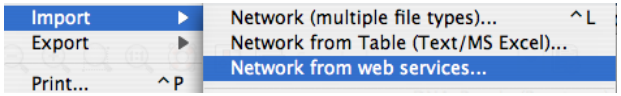
Filter/Query

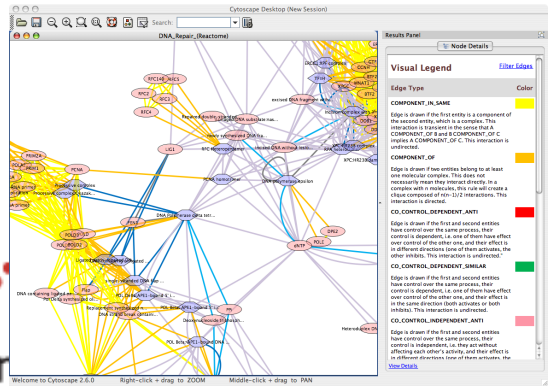


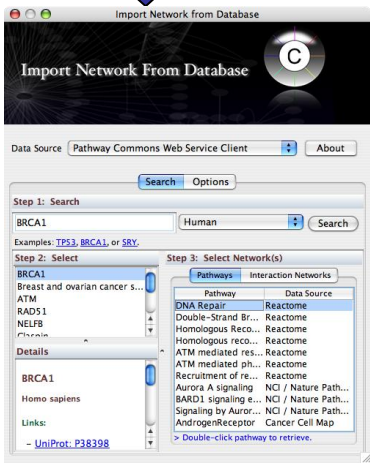
Automatic Layout



Interaction Database Search

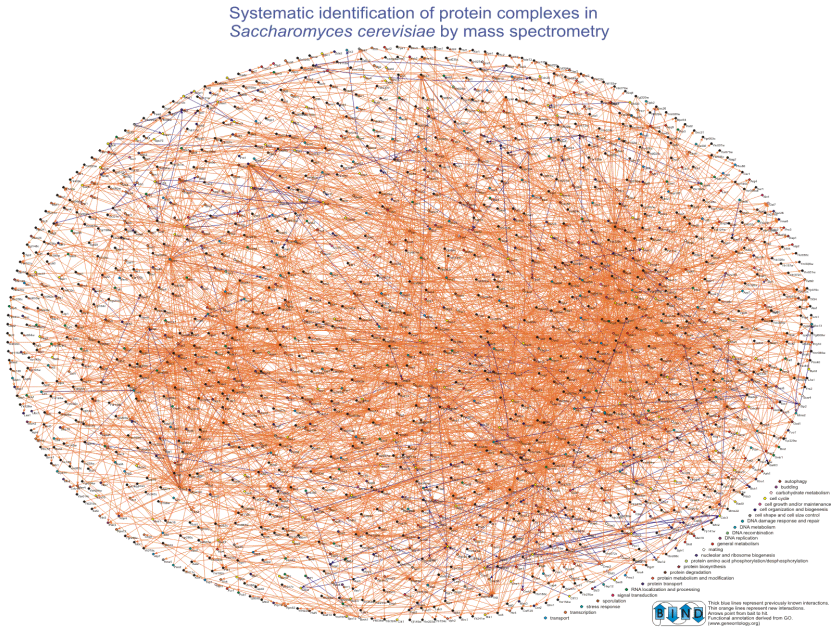






Module 1: Gene List/Network Intro

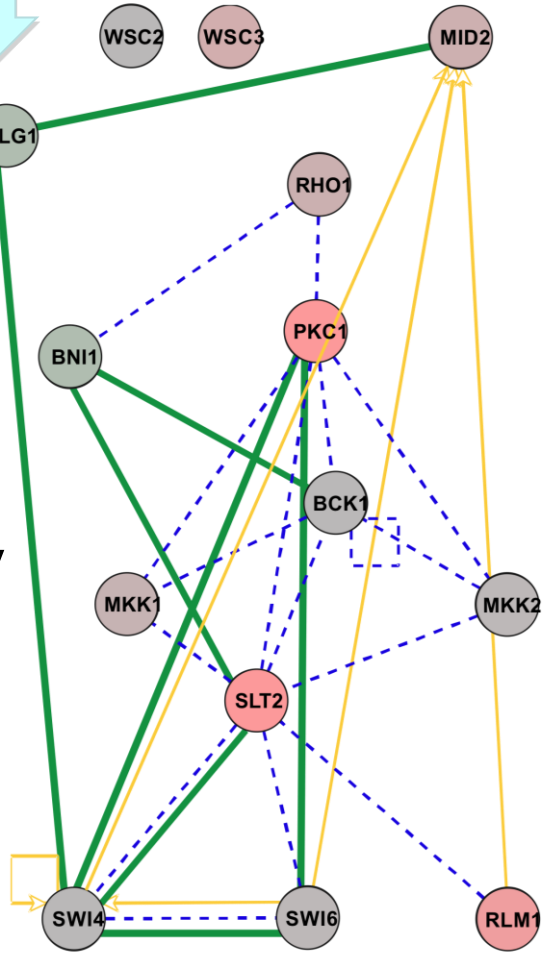
Overview



Zoom

Focus

PKC Cell Wall Integrity



Synthetic Lethal

Transcription Factor Regulation

Protein-Protein Interaction

Up Regulated Gene Expression

Down Regulated Gene Expression

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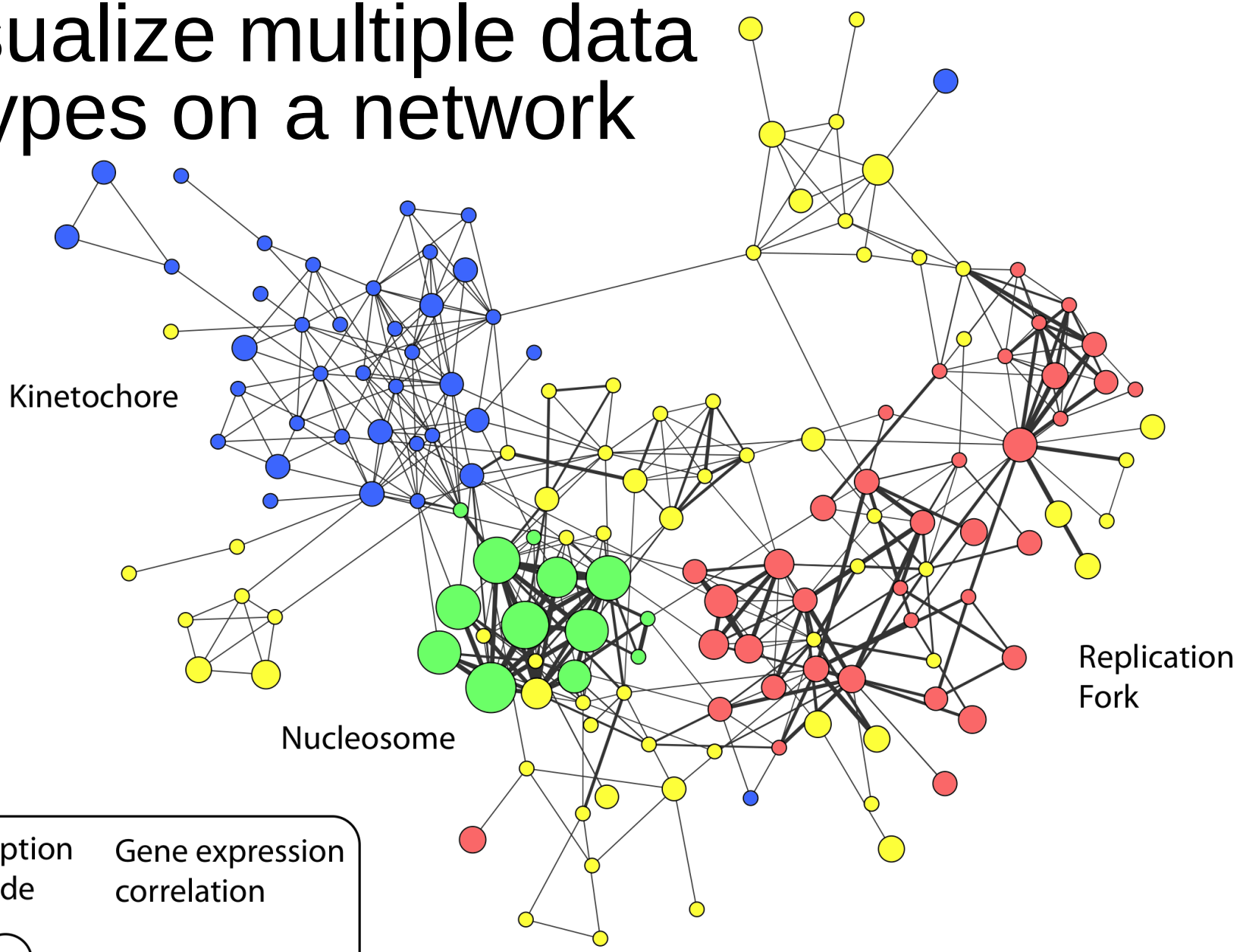
Visualize multiple data types on a network



Control: node/edge size, shape, color...

Active Community

http://www.cytoscape.org

- Help
  - 8 tutorials, >10 case studies
  - Mailing lists for discussion
  - Documentation, data sets
- Annual Conference: Houston Nov 6-9, 2009
- 10,000s users, 2500 downloads/month
- >40 Plugins Extend Functionality
  - Build your own, requires programming

Cline MS et al. Integration of biological networks and gene expression data using Cytoscape Nat Protoc. 2007;2(10):2366-82

What Have We Learned?

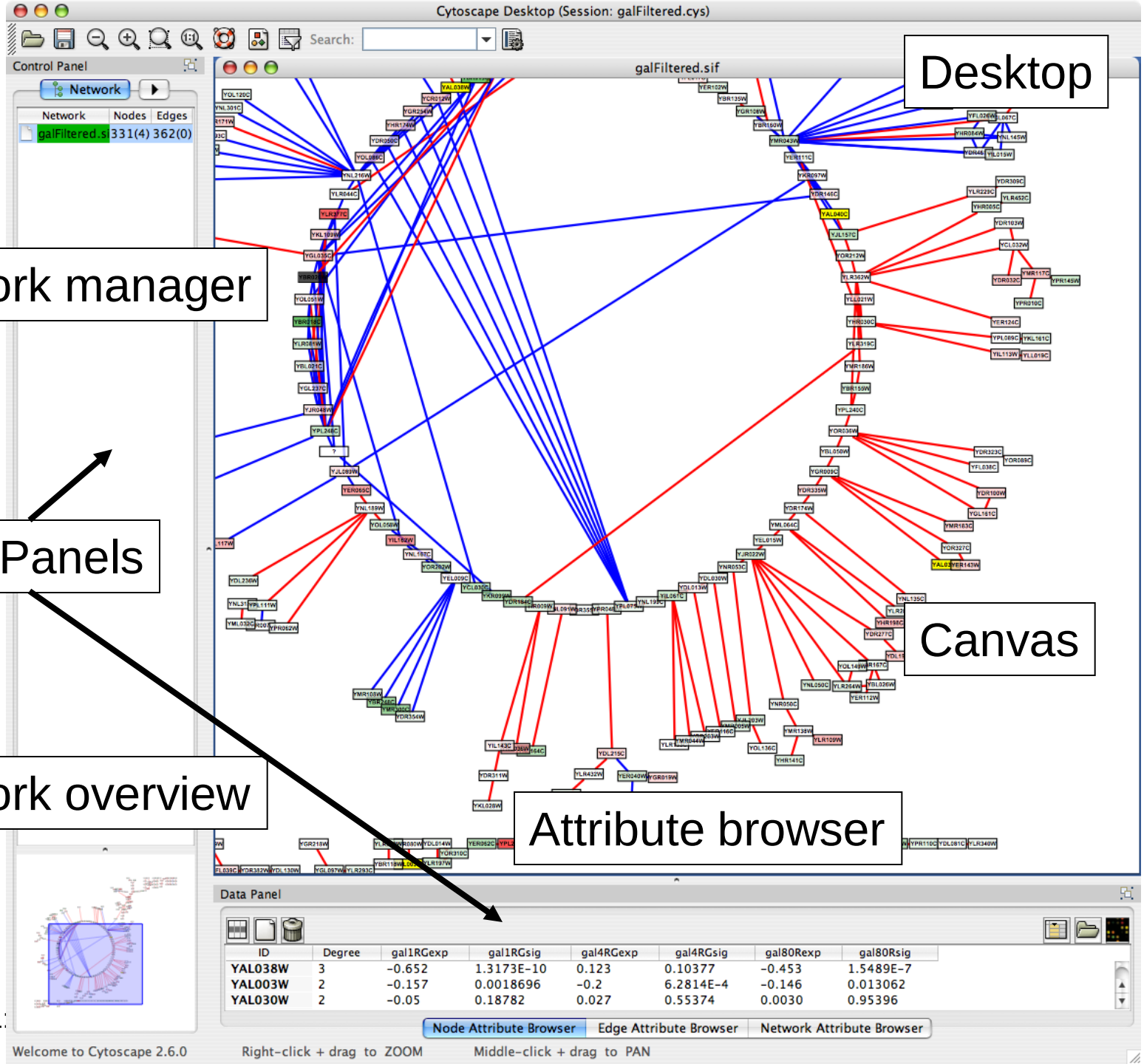
- Cytoscape is a useful, free software tool for network visualization and analysis
- Provides basic network manipulation features
- Plugins are available to extend the functionality

Cytoscape Demo

Version 2.6

www.cytoscape.org

FYI



Desktop

Network manager

CytoPanels

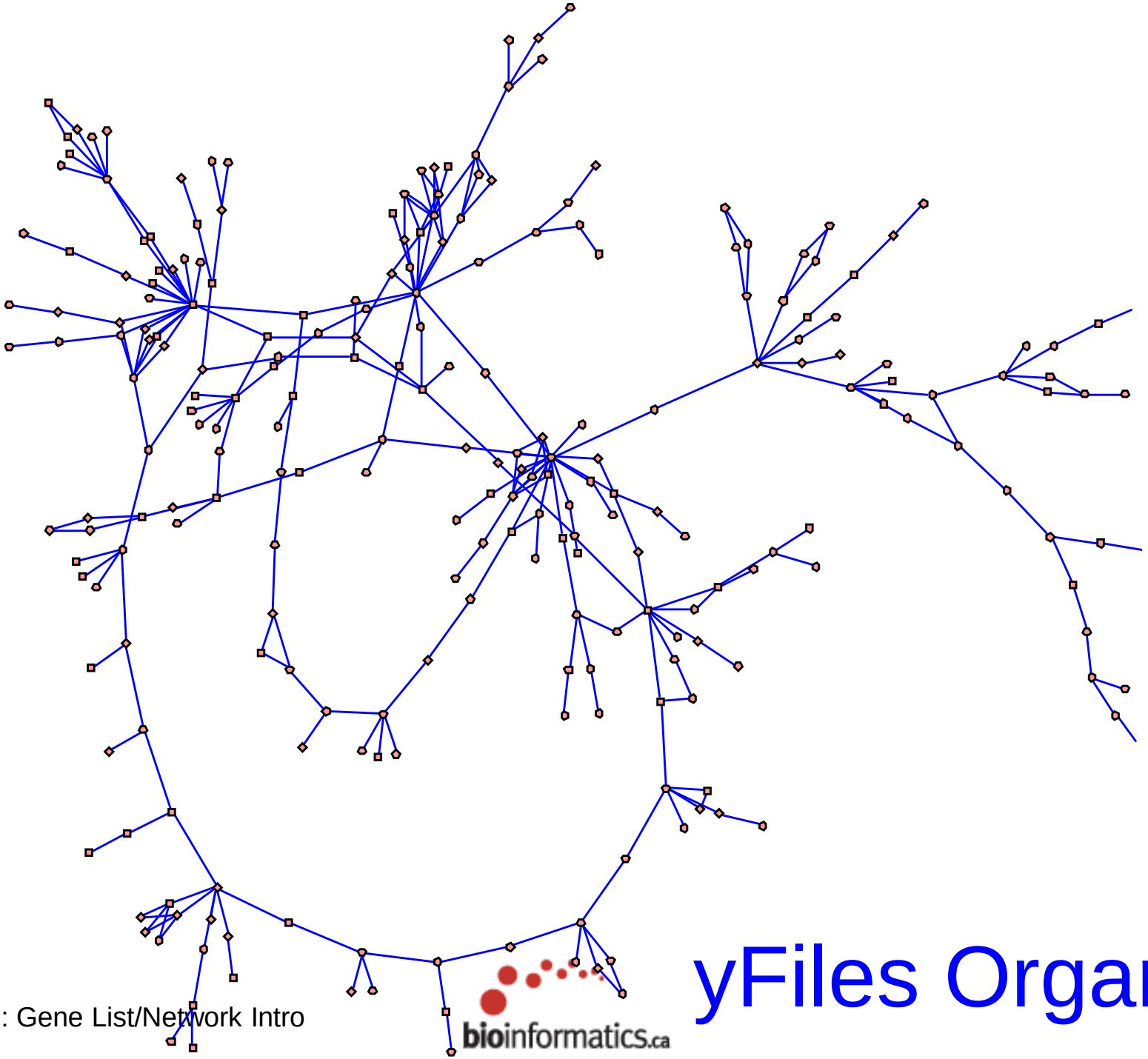
Canvas

Network overview

Attribute browser

Module 1

FYI



yFiles Organic

Module 1: Gene List/Network Intro

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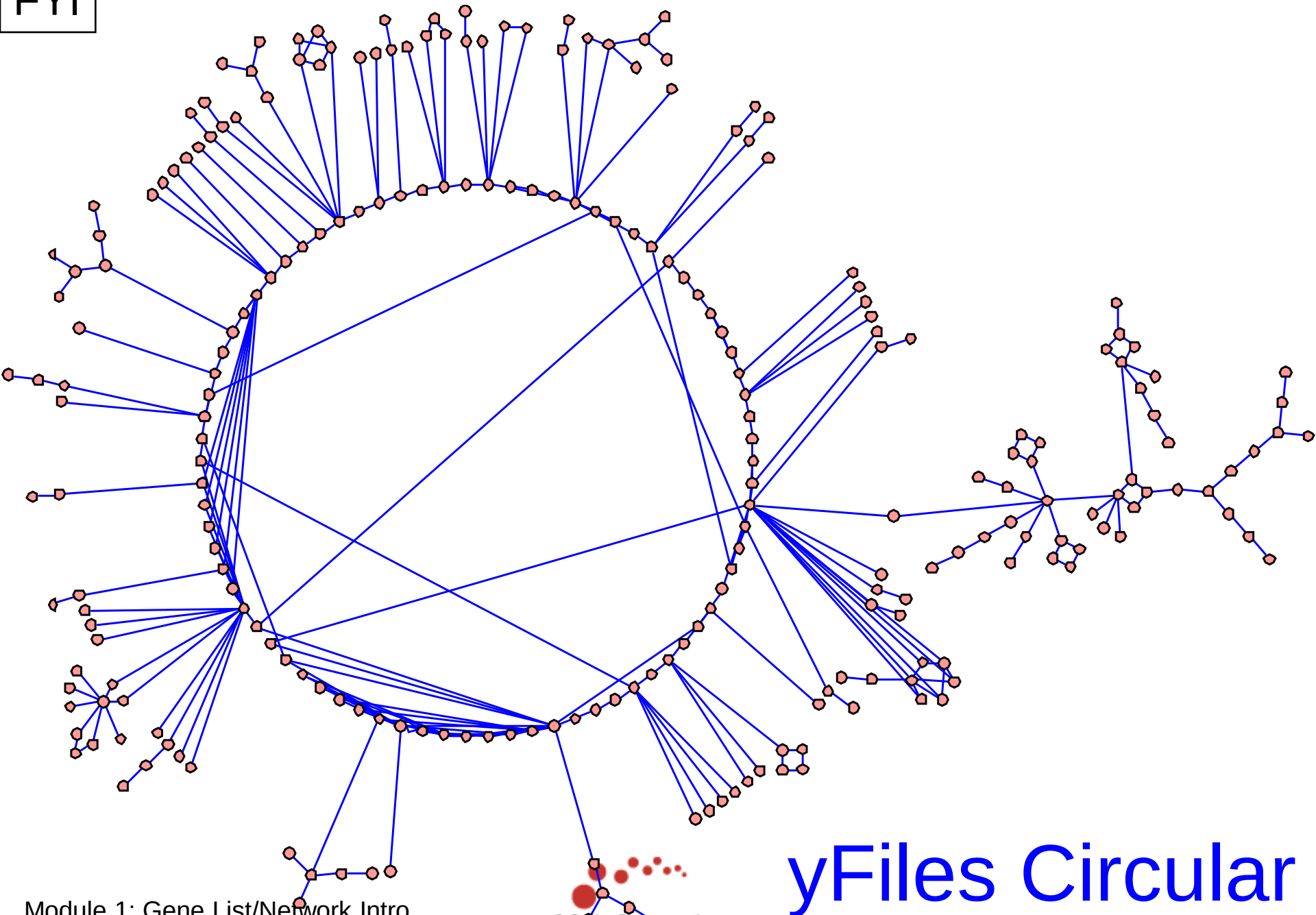
FYI



yFiles Circular

Module 1: Gene List/Network Intro

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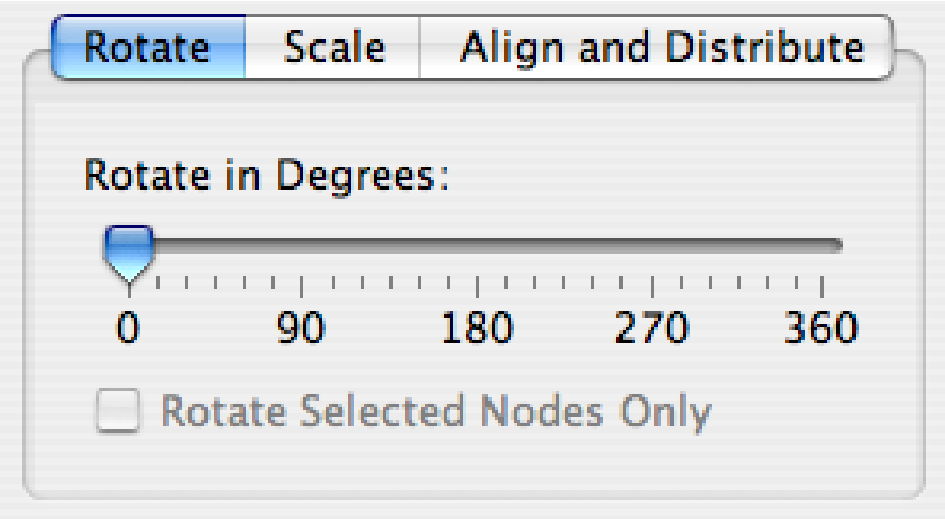
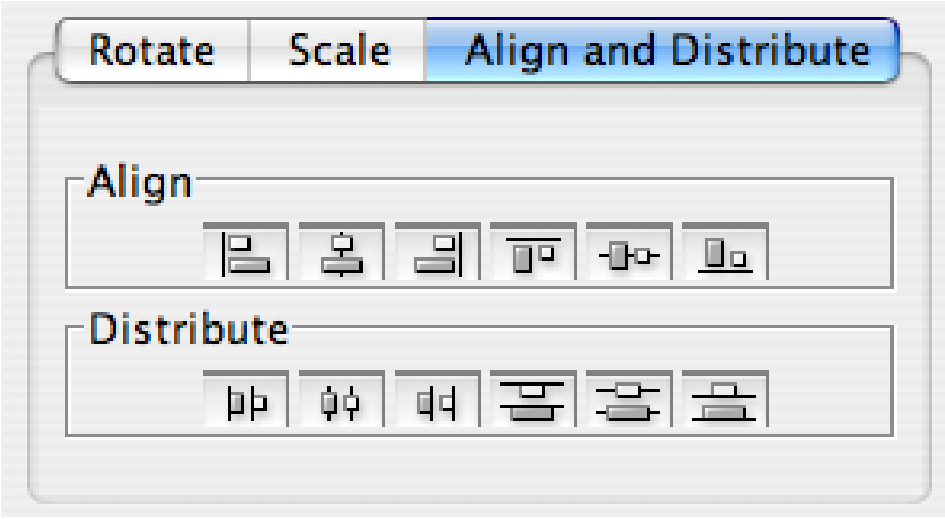
FYI

Network Layout

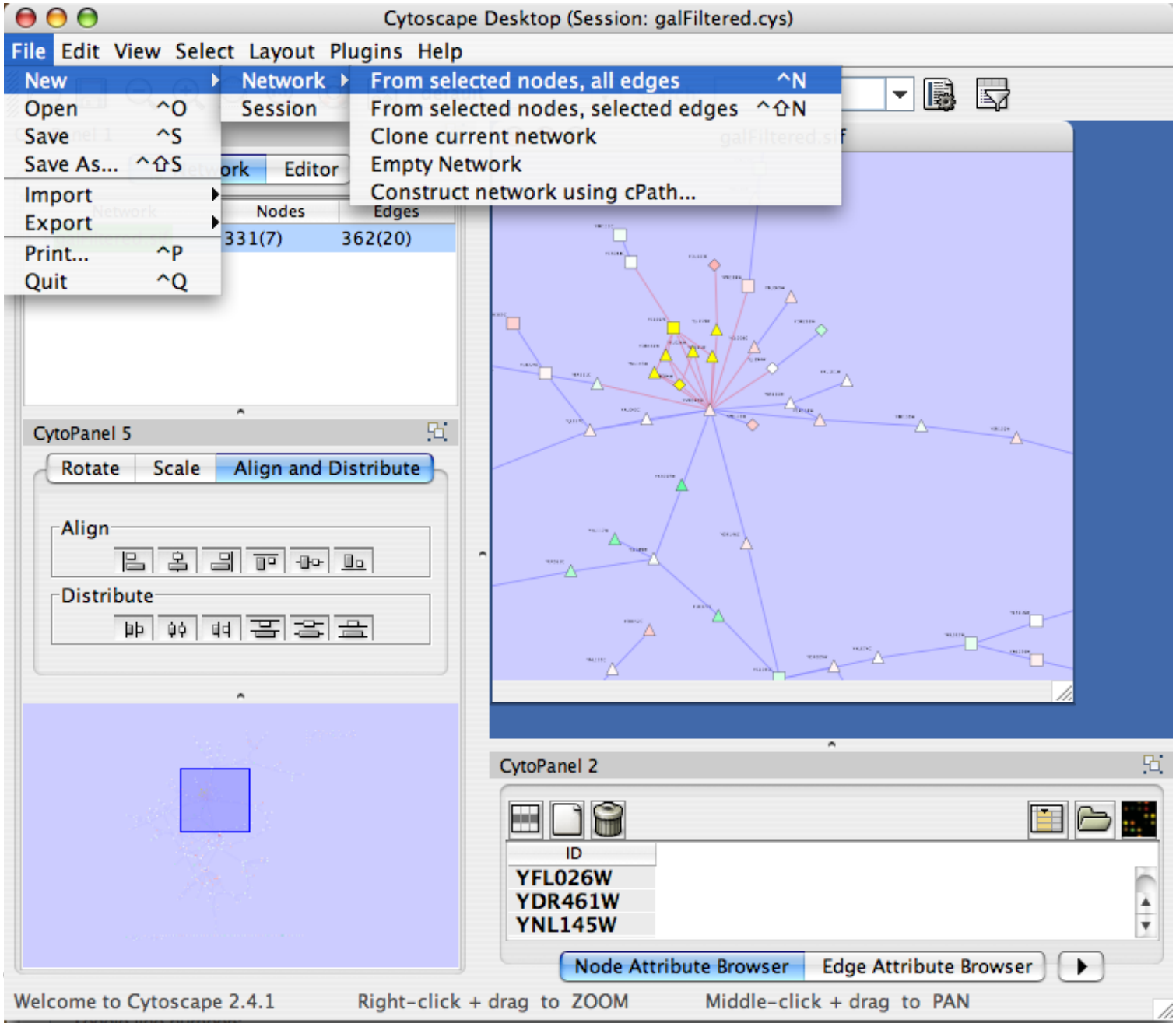
- 15 algorithms available through plugins
- Demo: Move, zoom/pan, rotate, scale, align





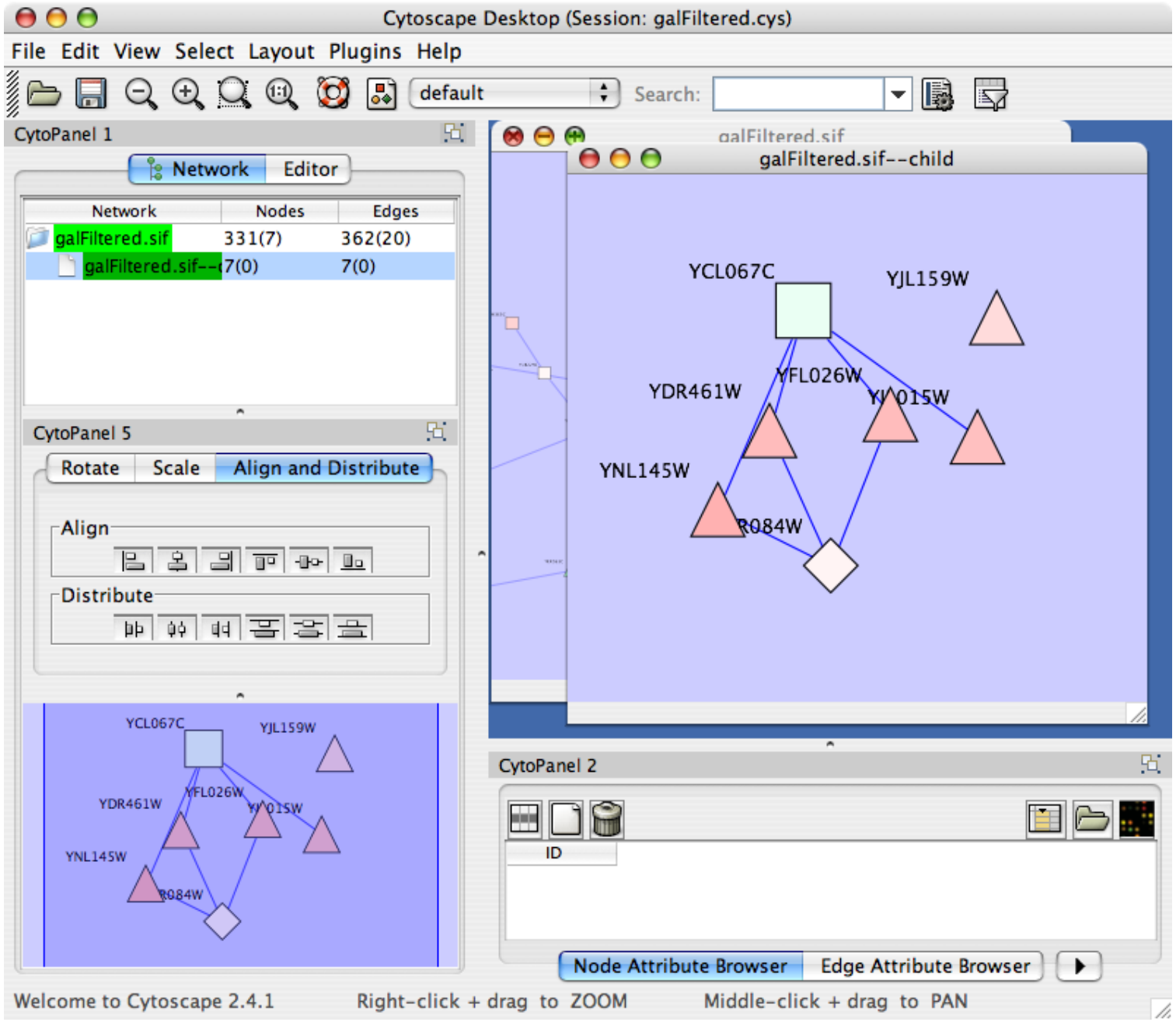
FYI

Create Subnetwork



FYI

Create Subnetwork



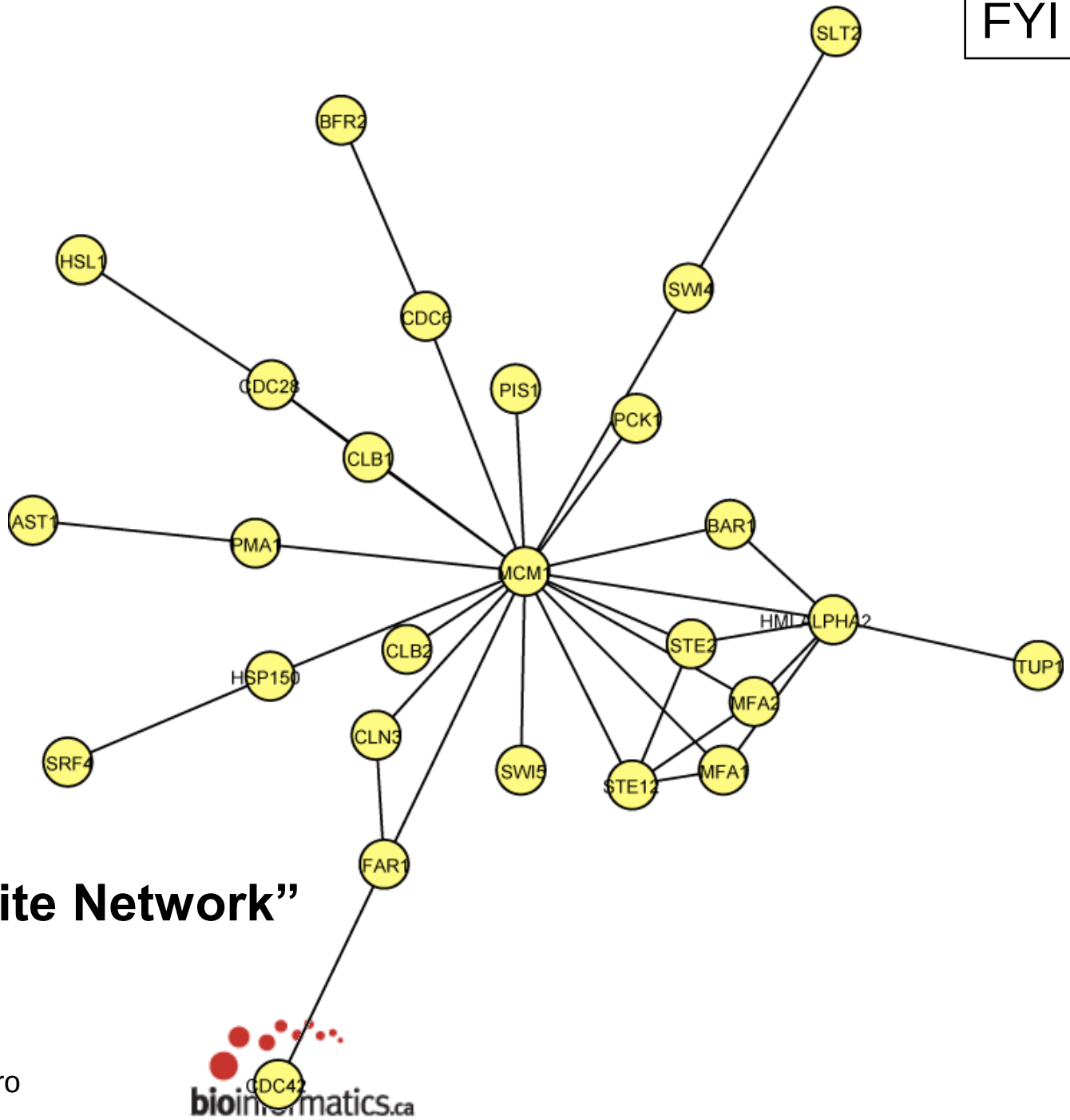
FYI

Visual Style

- Customized views of experimental data in a network context
- Network has node and edge attributes
  - E.g. expression data, GO function, interaction type
- Mapped to visual attributes
  - E.g. node/edge size, shape, colour…
- E.g. Visualize gene expression data as node colour gradient on the network

FYI

Visual Style



Load “Your Favorite Network”

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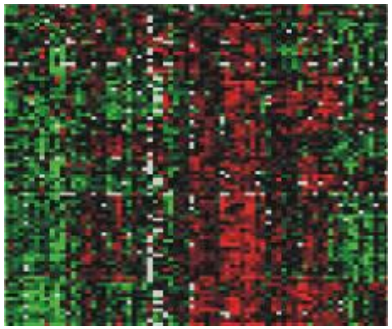
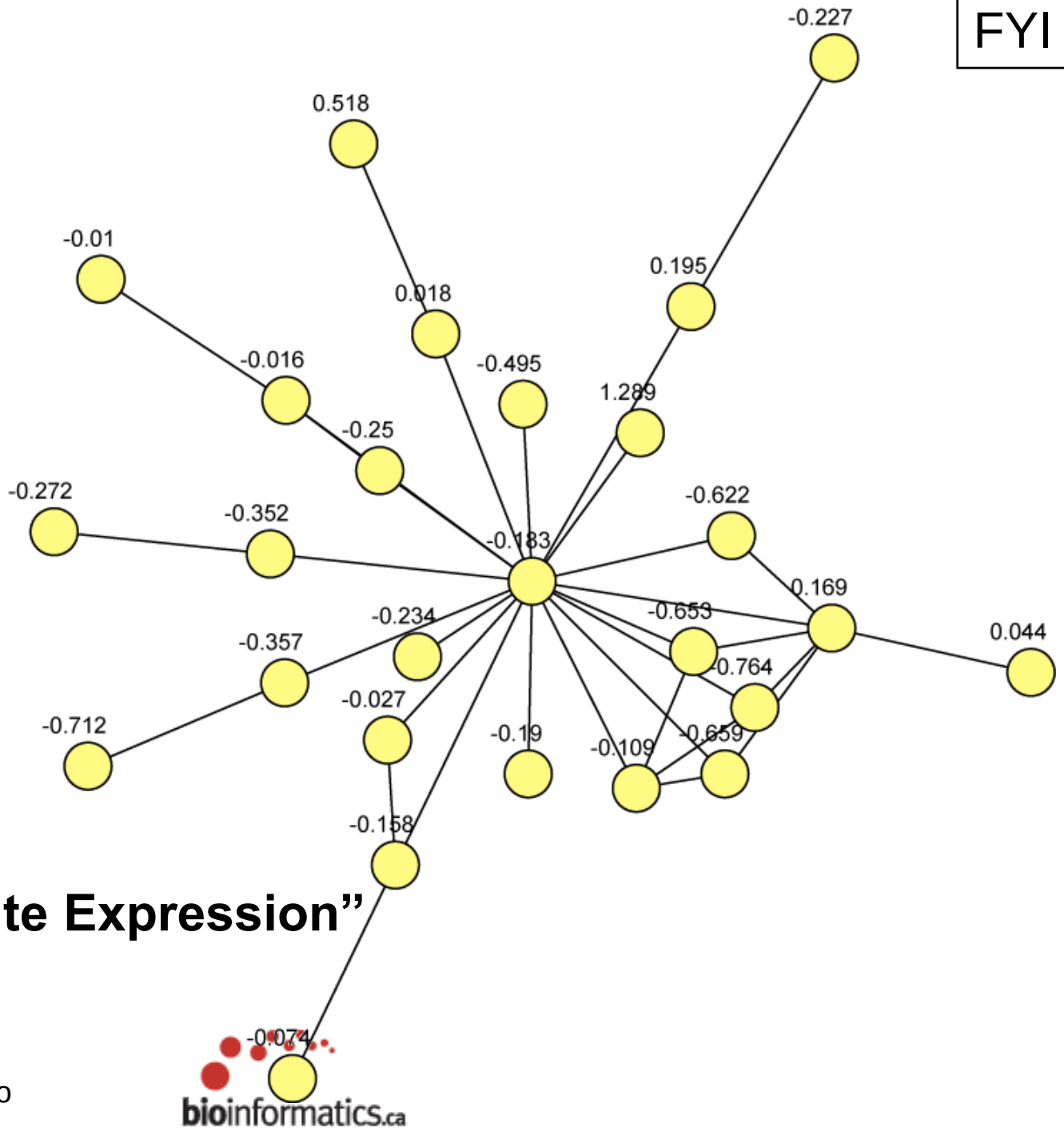
Visual Style

FYI





Load "Your Favorite Expression" Dataset

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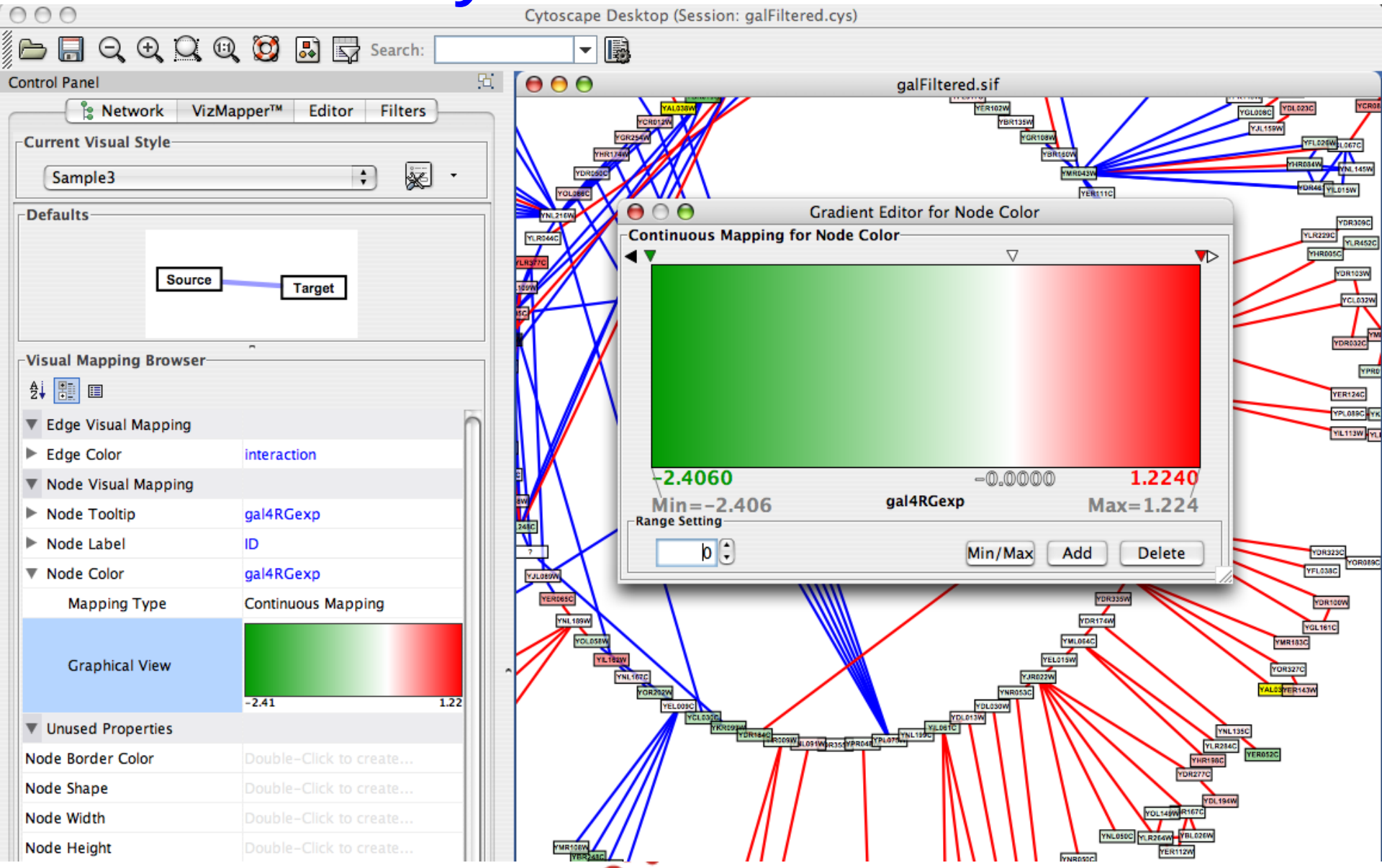
FYI

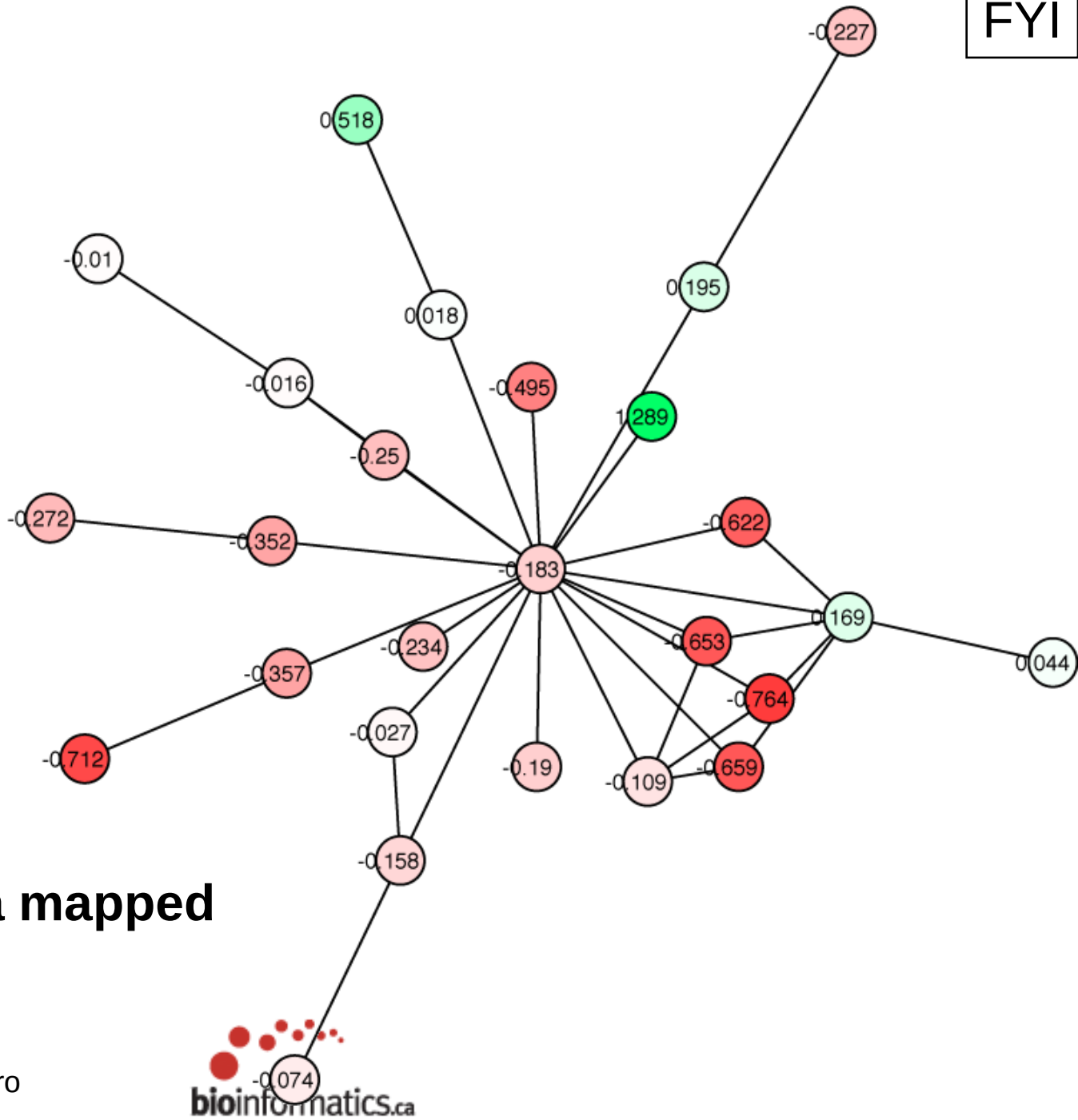
Visual Style



Map expression values to node colours using a continuous mapper

Visual Style

FYI



Expression data mapped to node colours

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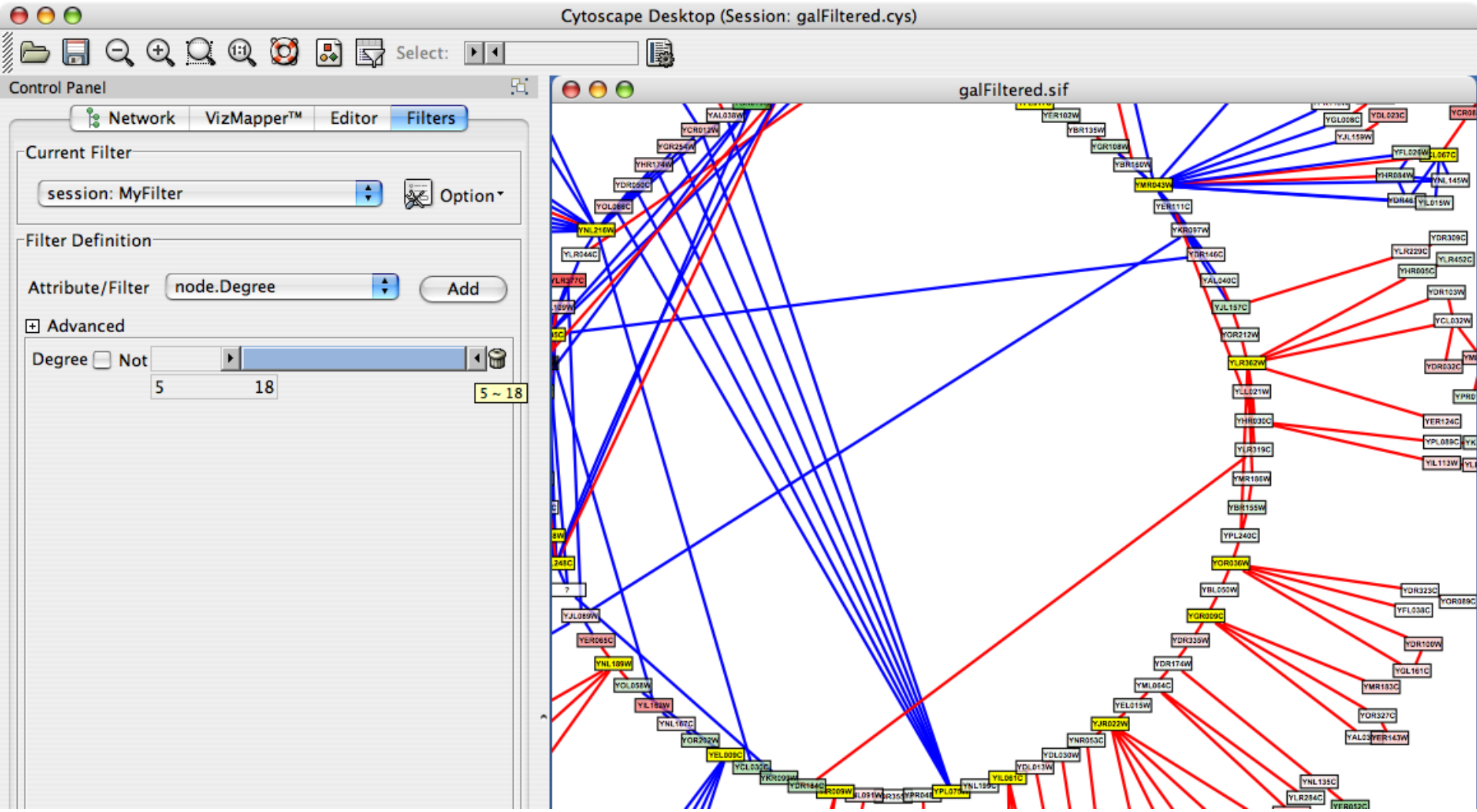
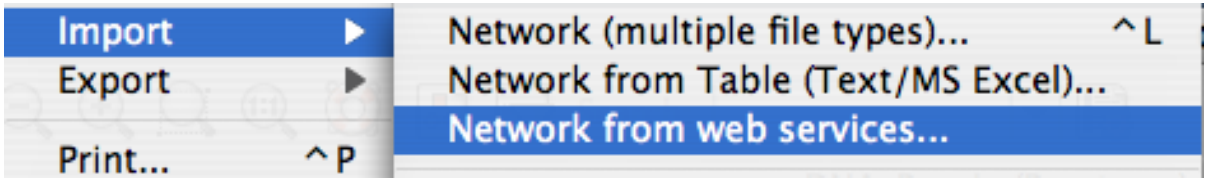
FYI

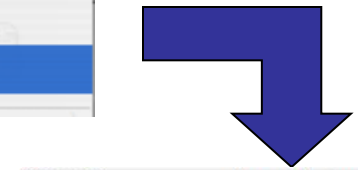
Network Filtering

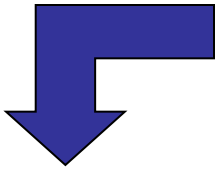


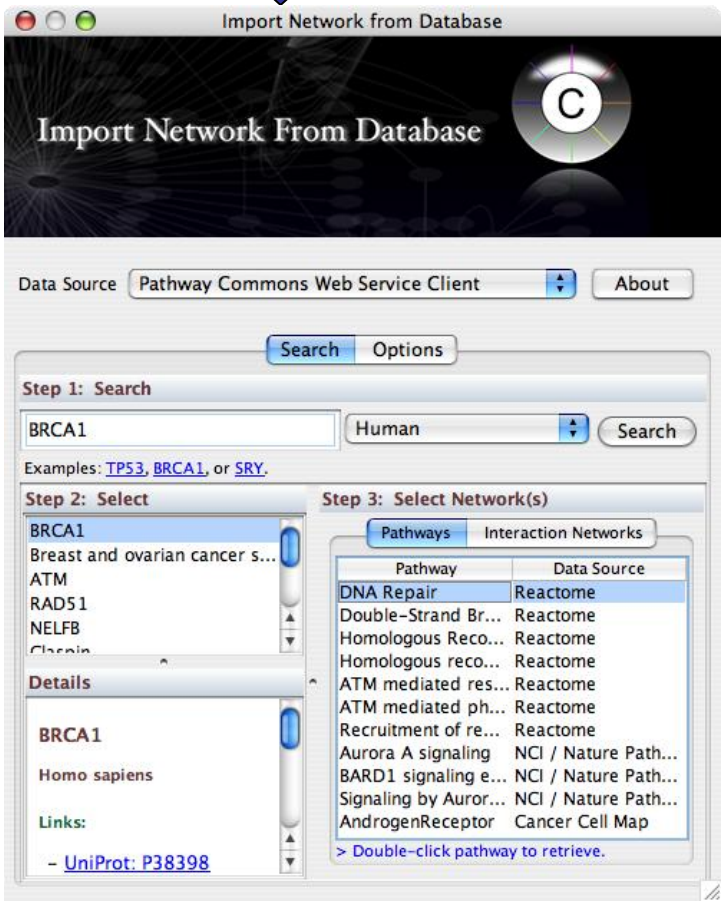
FYI

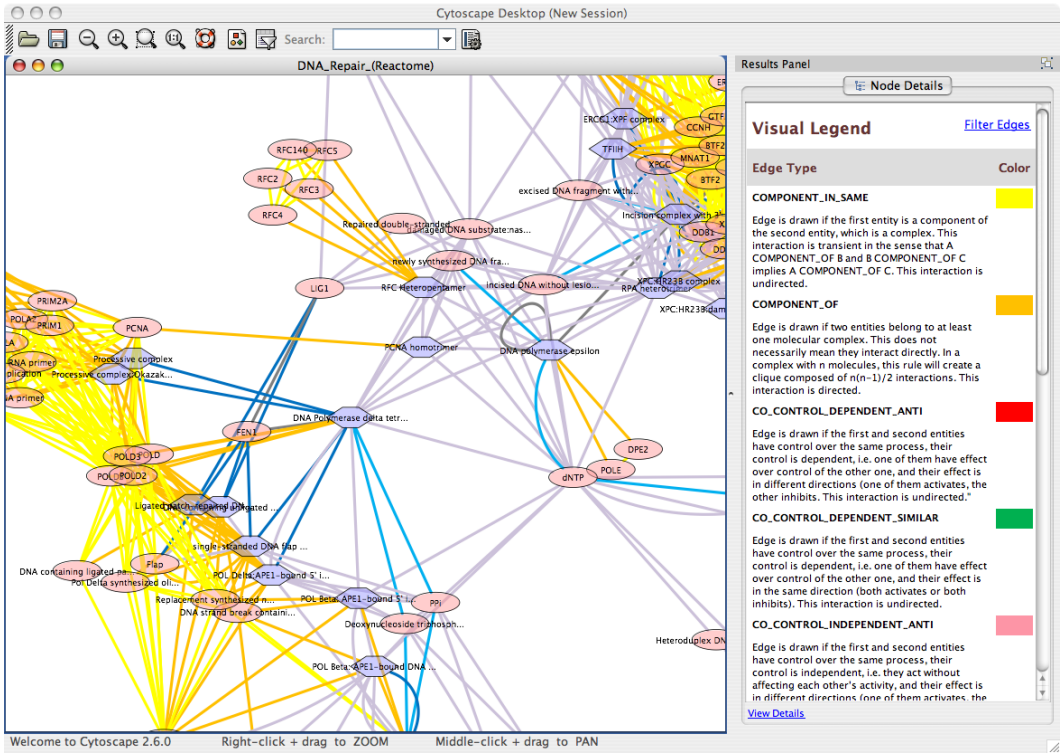
Interaction Database Search







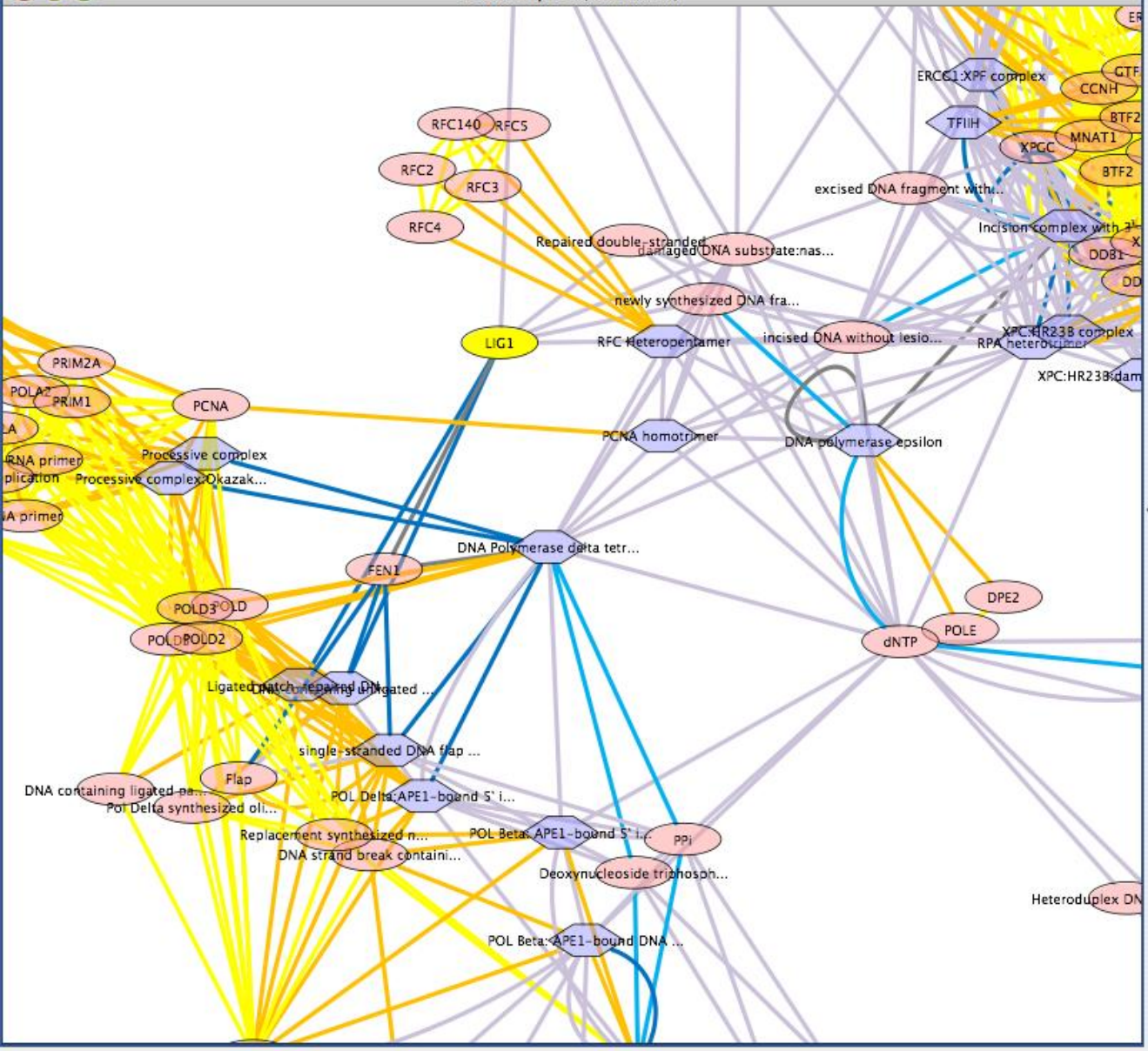




Cytoscape Desktop (New Session)

Search:

DNA_Repair_(Reactome)



Results Panel

Node Details

LIG1

Protein

Homo sapiens

Pathway Commons: 6311

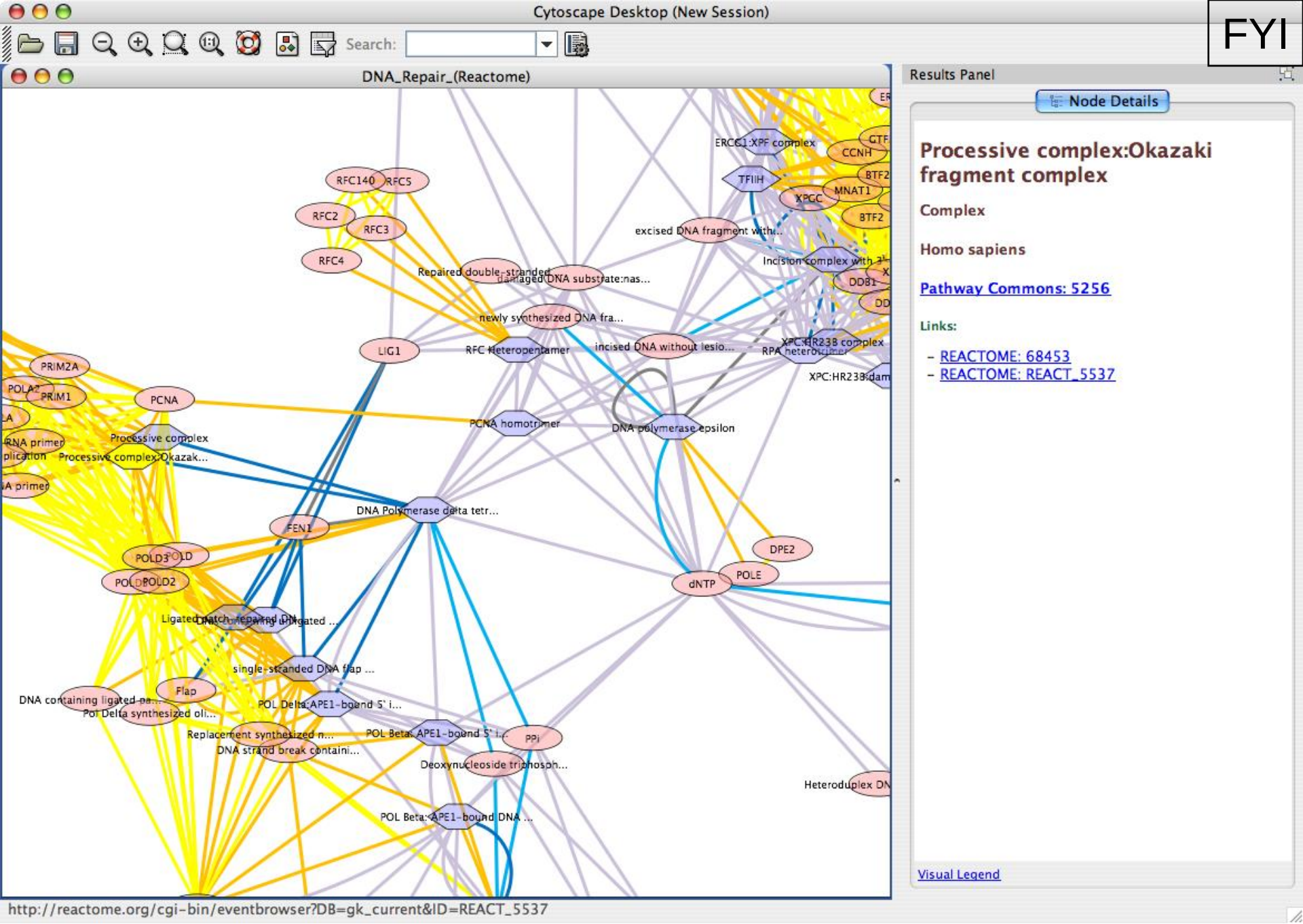
Synonyms:

- LIG1

Links:

- UNIPROT: P18858
- UNIPROT: Q32P23
- REF_SEQ: NP_000225
- Search iHOP

Visual Legend


Cytoscape Desktop (New Session)
Search:
DNA_Repair_(Reactome)
RFC140
RFC5
RFC2
RFC3
RFC4
Repaired double-stranded
damaged DNA substrate:nas...
newly synthesized DNA fra...
LIG1
RFC Heteropentamer
incised DNA without lesio...
ERCC1:XPF complex
CCNH
TFIIH
MNAT1
XPCC
excised DNA fragment with...
Incision complex with 3'
DDB1
XPC:HR23B complex
RPA heterotrimer
XPC:HR23B:dam
PRIM2A
PRIM1
PCNA
PCNA homotrimer
DNA polymerase epsilon
Processive complex
Processive complex:Okazak...
DNA Polymerase delta tetr...
FEN1
POLD3
POLD2
DPE2
POLE
dNTP
Ligated
single-stranded DNA flap ...
Flap
DNA containing ligated
Pol Delta synthesized oli...
POL Delta:APE1-bound 5' i...
Replacement synthesized n...
POL Beta:APE1-bound 5' i...
PPi
DNA strand break containi...
Deoxynucleoside triphosph...
Heteroduplex DN
POL Beta:APE1-bound DNA ...
Results Panel
Node Details
Processive complex:Okazaki fragment complex
Complex
Homo sapiens
Pathway Commons: 5256
Links:
REACTOME: 68453
REACTOME: REACT_5537
Visual Legend
http://reactome.org/cgi-bin/eventbrowser?DB=gk_current&ID=REACT_5537
FYI